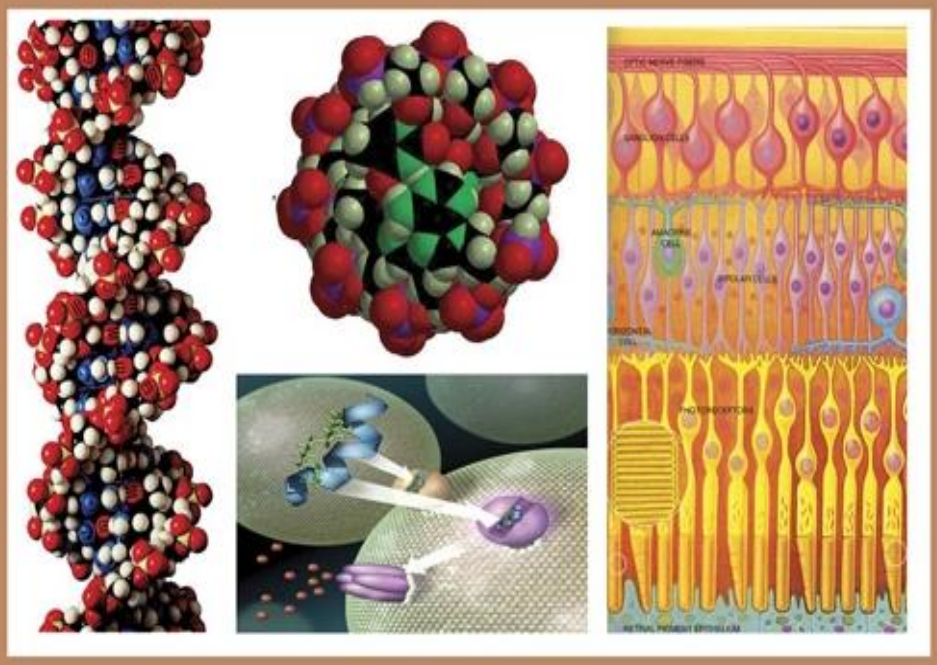
EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

C



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 13 No. 2 (2021)

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 13(2) pp113-127 (2021)*

DOI: 10.21608/EAJBSC.2021.198982

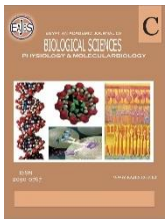
Egypt. Acad. J. Biolog. Sci., 13(2):113-127 (2021)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg



Antioxidant and anti-Inflammation Potentials of *Moringa oleifera* Aqueous Extract Against breast cancer chemotherapy drug Induced Kidney and Liver Toxicity in Experimental Rats

Essawy A.[1], Beeker H.M.[1], Ola N. Sayed [2]Abdel-Wahhab K.G.[3] and Saber S.R.[2]

1-Chemistry Department, Fayoum University, Fayoum, Egypt

2-Biochemistry Department, Fayoum University, Fayoum, Egypt

3- Medical Physiology Department, National Research Centre, Cairo, Egypt

*E. Mail : ons00@fayoum.edu.eg

ARTICLE INFO

Article History

Received:27/8/2021

Accepted:6/10/2021

Keywords:

Moringa oleifera; Tamoxifen; Liver; ALT, AST, TNF-α

ABSTRACT

Breast cancer is the most common causing death in women. Tamoxifen, 1-[4-(2-dimethyl-aminoethoxy) phenyl]-1,2-diphenyl-1-butene), is a nonsteroidal antiestrogen breast cancer prevention drug causing liver toxicity. This study was carried out to elucidate the efficacy of *Moringa oleifera* aqueous extract against carcinogenic effect of Tamoxifen in male rats. 50 male albino rats were divided equally into five groups: control group (I); *Moringa oleifera* aqueous group (II) of dose (300mg/Kg/day for 6 weeks); group (III) received Tamoxifen (3mg/Kg/3 days for 6 weeks); rats received orally Tamoxifen and *Moringa oleifera* aqueous extract together for 6 weeks group (IV) and rats pretreated with *Moringa oleifera* aqueous extract then Tamoxifen with the same dose and period group (V). In group (III) there were elevate in serum activities of ALT, AST, LDH, and serum levels of TNF-α, IL-1β, Il-10, cholesterol, TG, LDL, HDL, Urea, Creatinine also liver MDA; NO; ATPase. There were decrease in serum Total protein, Albumin and liver antioxidants markers (TAC and GSH) compared to control. In conclusion, oral administration of *Moringa oleifera* aqueous extract improved the biochemical markers and provided antioxidant activity against the toxicity of Tamoxifen in liver tissues.

INTRODUCTION

Breast cancer is the second most common cancer worldwide and the leading cause of cancer death in women (Siegel *et al.*, 2012 and Tomao *et al.*, 2015). Approximately 85% of all breast cancers are hormonal. They can be treated with hormone therapies, including tamoxifen and aromatase inhibitors (AIs) as anastrozole (Arimidex), letrozole (Femara) and exemestane (Aromasin)(Anderson *et al.*, 2002).

Tamoxifen has antiestrogen used in the prevention and treatment of breast cancer for both men and women through block the binding of estrogen, such as 17b-estradiol, to its receptor (McDonnell, 1999; Johnston, 2005 and Deroo *et al.*, 2006).

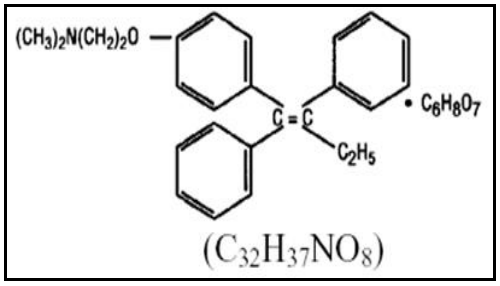


Fig.1: Chemical structure and empirical formula of Tamoxifen (Shoda *et al*., 2014).

Tamoxifen is metabolized in the liver by metabolizing enzymes into 4-hydroxytamoxifen (4-OHT) and endoxifen which have 100 times more affinity for the estrogen receptor than tamoxifen itself (Desta*et al*., 2004; Johnson *et al*., 2004 and Saladores *et al*., 2015). Tamoxifen has been shown to increase oxidative stress and induce apoptosis (Yang *et al*., 2013).

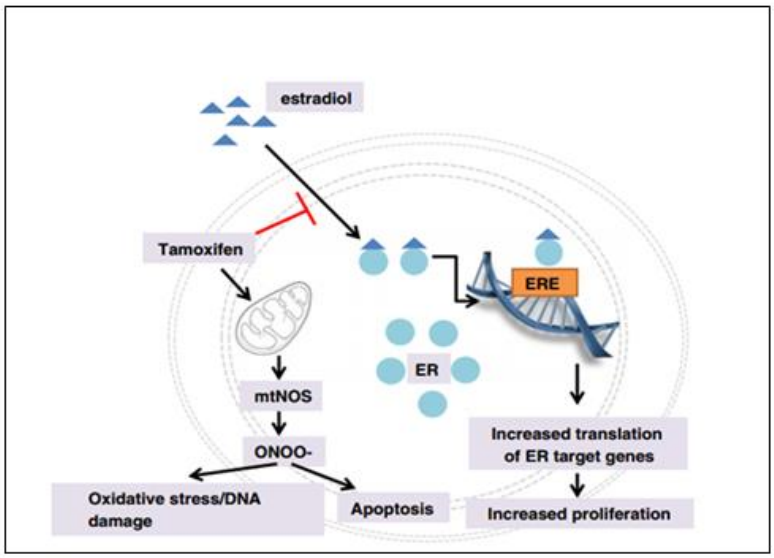


Fig.2: The mechanism of action for Tamoxifen (Yang *et al*., 2013).

It was revealed that tamoxifen in high dose cause oxidative liver damage as it had been elucidated to be a hepatocarcinogen in rats (Ahotupa *et al*., 1994; Caballero *et al*., 2001; Galeone *et al*., 2006 and Ribeiro *et al*., 2014).). It may be more toxic to liver because it has higher affinity to hepatic tissues than to any other tissues. A high frequency of p53 mutations was detected in hepatocarcinomas induced by tamoxifen exposure (Vancutsem *et al*., 1994).

Moringa oleifera, known as Moringa, is an Indian tree grows in Asia, South America and Africa (Palafox *et al*., 2012; Bhutada *et al*., 2016). It belongs to the *Moringaceae* family, enriched with effective flavonoids such as kaempferol, rhamnetin, chlorogenic acid, rutin, apigenin which exhibited anti-inflammatory, antimicrobial, anti-cancer and anti-diabetic effects (Karthivashan *et al*., 2016 and José *et al*., 2020). It provided a hepatoprotective effect (Upadhyay *et al*., 2015).

The objective of that study is to evaluate the improvement effect of *Moringa oleifera* extract against hepatotoxicity of tamoxifen in male rats.

MATERIALS AND METHODS

Adult fifty male Wistar albino rats (120-150g) were obtained from Animal

House, National Research Centre, Giza, Egypt. The animals were housed in suitable plastic cages for one week to the new room condition. Excess and fresh tap water and standard rodent food pellets were always available. All animals were received human care in compliance with the standard institutional criteria for the care and use of experimental animals.

After the animals being acclimatized with experimental room conditions, they were divided randomly into five groups (10 animals each) as:

Group I) comprised of normal animals fed diet and acting as control.

Group II) Animals fed diet and subjected to daily oral administration of *Moringa oleifera* aqueous extract (300mg/Kg/day) for consecutive six weeks (Jaiswl *et al.,* 2009).

Group III) comprised of normal animals fed diet and subjected to oral administration of Tamoxifen (3mg/Kg/3days) for consecutive six weeks (pala *et al*., 2015)

GroupIV) rats received orally aqueous extract of *Moringa oleifera* (300mg/kg/day) in combination with Tamoxifen (3mg/kg/3days) for consecutive six weeks.

Group V) rats treated daily via oral administration of *Moringa oleifera* aqueous extract (300mg/kg/day) for consecutive six weeks before they were intoxicated orally with Tamoxifen (3mg/kg/3days) for a similar period.

Blood Sampling:

At the end of the study period, animals fasted overnight, and following diethyl ether anesthesia, blood specimens (3-7 ml) were drawn from the retro-orbital plexus using non-heparinized blood was withdrawn into open vacutainer collecting tubes (Paget & Thosmon, 1979). Blood specimens were centrifuged at 3000 rpm for 10 minutes using centrifuge to separate sera and then stored at -70°C until biochemical measurements.

Tissue Sampling:

After blood collection, all animals were rapidly sacrificed then liver and kidney of each animal were dissected and washed with saline, dried, rolled in a piece of aluminum foil and stored at –70 °C until homogenization and biochemical determinations.

Preparation of Liver and Kidney Homogenate:

A specific weight from each organ (liver or kidney) was subjected to homogenization in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v) and dilution factor equal 10**;** then homogenate was centrifuged at 9000 rpm for 20 min to remove the nuclear and mitochondrial fractions. The supernatant was divided into aliquots and stored at -70°C till the determination of the biochemical measurement.

Aqueous Extract of *Moringa oleifera*:

Moringa (*Moringa oleifera*) aqueous extract was carried out according to the method of Berkovich *et al.* (2013). 50 g of the dry-powdered leaves were mixed with 500 ml boiling distilled water for 15minutes. The mixture was then filtered through sterile Whatman filter paper number 42 using Buchner funnels. The filtrate was subjected to lyophilization process through freeze drier under pressure, 0.1-0.5 m Bar and temperature -35-41^{0}C conditions. The dry extract was stored in a dark bottle at -20^{0}C.

Histo-Pathological Examination:

The livers and kidneys from each animal of the study groups were fixed in 10% formalin-saline buffer for 24 hours; then washing in tap water followed by dehydration in graded alcohol, clearing in xylene for 20 minutes and embedded in paraffin wax. Transverse serial sections were then cut at 5 micrometers thickness and mounted on albumenized slide. Sections were stained with hematoxylin and eosin then investigated (Drury & Wallington, 1980).

Biochemical Analysis:

Liver Function Biomarkers Assays:

ALT, AST and LDH activities

were evaluated according to Schumann and Klauke (2003) and Tietz(1983) respectively. Total protein and albumin were performed according to Berth and Delanghe (2004).

Kidney Function Biomarkers Assays:

Creatinine and Urea were evaluated according to the method of Allen (1982) and Eisenwiener (1976) respectively.

Oxidative Stress Biomarkers Assays:

Lipid peroxidation was performed by measuring MDA levels in the supernatant of liver and kidney homogenate according to (Draper & Hadley, 1990).

ATPase activity was measured according to the chemical method described by Tsakiris *et al*. (2004). Nitric oxide level was determined according to the method of Montgomery and Dymock (1961).

Total antioxidant capacity and Glutathione level of tissue homogenate determined according to the method of Koracevic *et al.* (2001) and Beutler *et al.* (1963) respectively.

Anti-Inflammatory Biomarkers Assays:

Serum levels of IL-1ß; IL-10 and TNF-α were determined using Enzyme-Linked Immunosorbent Assay technique with ELISA kit as described by (Allan*et al*., 2005 and Brouckaert *et al.* (1993) respectively.

Lipid Biomarkers Assays*:*

Serum total cholesterol level; triglycerides; HDL and LDL were determined according to the photometric system described by Berth and Delanghe(2004) and Naito (2003) respectively.

Statistical Analysis:

The obtained data were subjected to one-way ANOVA followed by a post hoc test (Duncan). All data were expressed as mean ± standard error (SE). Differences were considered significant at $P \leq 0.05$.

RESULTS

This study was carried out to evaluate the ameliorative effect of *Moringa oleifera* aqueous extract against the toxicity of tamoxifen in male rats. The obtained data showed that daily oral administration of rats with moringa aqueous extract had no effect on the activities of serum ALT; AST and LDH (-2.34; -4.22 and -4.3%) respectively; while oral administration with Tamoxifen led to a significant increase (46.0; 77.17 and 42%, respectively) in their activities compared to control group. Animals treated orally with moringa in group IV showed a significant decrease in their activities than group V comparing with rats treated with tamoxifen (-32.95; -37.38 and -21.39%, respectively) (Table 1).

Our results showed that rats that received daily moringa extract resulted in non-significant changes (3.03 and 2.94% respectively) in serum total proteins and albumin while oral intoxication with Tamoxifen led to a significant decrease in their levels when compared with the control group (-10.60 and -14.70% respectively); while there improvement in their levels in group IV and group V (7.2; 3.6; 6.6 and 3.3 g/dl respectively) when compared with group III (Table 2).

There was a significant increase in creatinine and urea levels in rats of group III which are treated with tamoxifen when compared to control (40.84 and 112.28% respectively). Administration of moringa extract improved their levels in both group IV and group V but receiving moringa extract with tamoxifen effective than group V (Table 2).

Table 1: Mean values and percentage of change of serum ALT; AST and LDH activities in all studying groups.

Groups \ Parameters		ALT (IU/L)	AST (IU/L)	LDH U/L
Control (I)	M±SE	72.6±2.98	125.3±4.44	1162±55
MAE (II)	M±SE	70.9±2.87	120±4.24	1112±53
	% A	-2.34%	-4.22	-4.30
TMX (III)	M±SE	106.2±13.01[a]	222±21.45[a]	1650±78[a]
	%A	46.0%	77.17	42.00
MAE+TMX (IV)	M±SE	71.200±13.3[b]	139±8.09[b]	1297±61[b]
	% B	-32.95%	-37.38	-21.39
MAE→TMX(V)	M±SE	83.600±5.7[b]	180±11.81[ab]	1468±70[ab]
	% B	-21.28%	-18.91	-11.00

∗ All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
∗ MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
∗ %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.

Table 2: Mean values and percentage of change of serum Total.Proteins; Albumin; Creatinine and Urea levels in all studying groups.

Groups \ Parameters		Total Proteins g/dl	Albumin g/dl	Creatinine mg/dl	Urea mg/dl
Control (I)	M±SE	6.6±0.11	3.4±0.04	0.71±0.06	57±2.8
MAE (II)	M±SE	6.8±0.12[b]	3.5±0.04[b]	0.72±0.06[b]	53±2.8[b]
	%A	3.03	2.94	1.40	-7.02
TMX (III)	M±SE	5.9±0.18[a]	2.9±0.09[a]	1.0±0.08[a]	121±14.0[a]
	%A	-10.60	-14.70	40.84	112.28
MAE+TMX (IV)	M±SE	7.2±0.14[a]	3.6±0.05[b]	0.8±0.02[b]	92±11.0[ab]
	%B	22.0	24.14	-20.0	-23.96
MAE→TMX (V)	M±SE	6.6±0.11[ab]	3.3±0.032[ab]	0.9±0.07[ab]	108±12.8[ab]
	%B	11.86	13.79	-10.0	-10.74

∗ All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
∗ MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
∗ %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
∗ a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group .

The obtained data showed that daily oral administration of rats with moringa extract resulted in non-significant changes (1.17, -6.41&-2.56% respectively) in the concentration of serum total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-c), respectively with a non-significant increase (6.66%) in serum high-density lipoproteins cholesterol (HDL-c) level. In contrast, oral administration with Tamoxifen causes a significant increase (44.70,55.13&71.79% respectively) in the serum of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c) levels matched with a significant decrease (-33.33%) in serum high-density lipoproteins cholesterol (HDL-c) level compared to control group. (Table 3).

Animals treated with moringa extract with tamoxifen (group IV) showed a significant decrease (-22.76,-15.70&-

19.40% respectively) in serum total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-c) levels concomitant a significant raise (30.0%) in serum high-density lipoproteins cholesterol (HDL-c)level more than group V when compared with control group (Table 3).

Table 3: Mean values and percentage of change of serum Cholesterol; TG; LDL and HDL levels in all studying groups.

Parameters / Groups		Cholesterol mg/dl	TG mg/dl	LDL mg/dl	HDL mg/dl
Control (I)	M±SE	85±3.9	78±2.7	39±2.7	30±3.9
MAE (II)	M±SE	86±3.9	73±2.5	38±2.6	32±4.0
	% A	1.17	-6.41	-2.56	6.66
TMX (III)	M±SE	123±5.6^{a}	121±4.2^{a}	67±4.5^{a}	20±1.4^{a}
	%A	44.70	55.13	71.79	-33.33
MAE+TMX (IV)	M±SE	95±4.3^{a}	102±3.5^{b}	54±3.7^{ab}	26±2.6^{a}
	% B	-22.76	-15.70	-19.40	30.0
MAE→TMX (V)	M±SE	113±5.2^{ab}	116±3.4^{ab}	61±4.1^{ab}	18±0.8^{ab}
	% B	-8.13	-4.13	-8.95	-10.0

- All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
- MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
- %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
- a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group

Oral administration with Tamoxifen (group III) showed a significant elevation (75.47&34.76&24.46% respectively) in serum TNF-α, IL1β and IL 10 levels, respectively when compared with control. On the other side, animals treated with moringa aqueous extract in combination with Tamoxifen (groupIV) showed a significant decrease (-22.58&-21.98&-18.80% respectively) in their levels more than group V when compared with group III (Table 4).

Table 4: Mean values and percentage of change of serum TNF-α; IL 1β; IL 10 and levels in all studying groups.

Parameters / Groups		TNF-α ng/L	IL 1β ng/L	IL 10 ng/L
Control (I)	M±SE	53±3.9	837±2.4	188±0.8
MAE (II)	M±SE	52±3.9	811±2.4	183±0.8
	% A	-1.89	-3.11	-2.65
TMX (III)	M±SE	93±6.9^{a}	1128±3.2^{a}	234±0.9^{a}
	%A	75.47	34.76	24.46
MAE+TMX (IV)	M±SE	72±5.3^{a}	880±2.6^{ab}	190±0.8^{ab}
	% B	-22.58	-21.98	-18.80
MAE→TMX (V)	M±SE	89±6.6^{a}	1054±3.1^{b}	222±0.9^{a}
	% B	-4.30	-6.56	-5.13

- All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
- MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
- %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
- a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group

From our study rats treated with Tamoxifen showed a significant increase (43.0; 35.19; 37.73 and 30.18% respectively) in the concentration of hepatic and renal MDA and NO levels with a significant decrease in ATPase activity (-28.73 and -26.95% respectively) when compared with control group. Comparing with Tamoxifen-intoxicated group, animals treated orally with moringa aqueous extract in combination with Tamoxifen (group IV) showed a significant decrease (-22.88, -21.92, -20.55 and -27.54% respectively) in the levels of hepatic and renal MDA and NO coupled with a significant increase (23.32 and 26.48% respectively) in ATPase activity better than group V (Tables 5 &6).

In group III where rats treated with tamoxifen showed a significant decrease in hepatic& renal TAC and GSH levels (-35.7;-23.8; -36 and -15.52%, respectively) when compared with the control group with improvement in their levels in groups IV &V (Tables 5&6).

Table 5: Mean values and percentage of change of ATPase; MDA; NO; TAC and GSH levels in liver homogenate of all studying groups:

Groups \ Parameters		ATPase mmol pi /hr	MDA mmol/g	NO mmol/g	TAC mmol/g tissue	GSH mg/g tissue
Control (I)	M±SE	355±1.3^{A}	107±4.1^{C}	54±3.7^{C}	28±0.5^{A}	63±2.3^{B}
MAE (II)	M±SE	318±1.3^{A}	105±4.1^{C}	52±3.5^{C}	29±0.5^{A}	65±2.5^{B}
	% A	-10.42	-1.86	-3.70	3.6	3.2
TMX (III)	M±SE	253±1.0^{D}	153±5.9^{A}	73±5.0^{A}	18±0.3^{C}	48±1.7^{C}
	%A	-28.73	43.00	35.19	-35.7	-23.8
MAE+TMX (IV)	M±SE	312±1.2^{B}	118±4.5^{C}	57±3.9^{BC}	22±0.4^{B}	82±3.0^{A}
	% B	23.32	-22.88	-21.92	22.2	70.8
MAE→TMX (V)	M±SE	290±1.2^{C}	141±5.5^{AB}	69±4.6^{AB}	19 ±0.4^{C}	54±2.1^{C}
	% B	14.62	-7.84	-5.48	5.55	12.5

- All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
- MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
- %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.

Table 6: Mean values and percentage of change of ATPase; MDA; NO; TAC and GSH levels in kidney homogenate of all studying groups.

Groups \ Parameters		ATPase mmol pi /hr	MDA mmol/g	NO mmol/g	TAC mmol/g tissue	GSH mg/g tissue
Control (I)	M±SE	460±7.2	53±2.4	53±1.8	25 ±0.8	58±2.2
MAE (II)	M±SE	427±6.7	51±2.4	51±1.8	25.1±0.8	61±2.2
	% A	-7.17	-3.77	-3.77	0.4	5.17
TMX (III)	M±SE	336±5.3^{a}	73±3.4^{a}	69±2.4^{a}	16±0.5^{a}	49±1.8^{a}
	%A	-26.95	37.73	30.18	-36.0	-15.52
MAE+TMX (IV)	M±SE	425 ±6.7^{ab}	58±2.7^{b}	49±1.7^{b}	19±0.6^{b}	74±2.6^{b}
	% B	26.48	-20.55	-27.54	18.75	51.0
MAE→TMX (V)	M±SE	386±6.1^{b}	64±3.0^{b}	62±2.1^{b}	17.9±0.5^{b}	55±2.1^{ab}
	% B	14.88	-12.33	-10.14	11.87	12.24

- All data are expressed as mean ± standard error (M±SE); each group of 10 animals (n=10).
- MAE (Moringa aqueous extract) and TMX (Tamoxifen drug).
- %A (percent of change calculated from control group) and % B is percent of change calculated from Tamoxifen drug group.
- a Significant at P value ≤ 0.01 versus control group and b Significant at P value ≤ 0.01 versus Tamoxifen group.
-

The liver sections of normal showed histological structure of normal hepatic lobules and central vein and the cell corda were separated by narrow blood sinusoids lined by endothelial cells and kupffer cells**.** Liver sections of the rats treated with moringa aqueous extract only showed liver tissue more or less appeared normal (Figs. 3A&B).

The microscopic examination of liver sections of rats treated with Tamoxifen only demonstrated dilated and congested portal vessels. Dilated bile duct and massive cellular infiltration around it. Dilated blood sinusoid could be observed. Sings of degeneration in the form of pyknosis, karyolysis and fibrosis were seen. Hypetrophy of kupher cells and vacuolar degeneration (Figs. 3C&D).

The microscopic examinations of liver sections of rats treated with tamoxifen along with moringa extract at the same time (group IV) induced an improvement in pathological changes in the form of no fibrosis, no inflammatory infiltrate, no signs of degeneration. Also, congestion of central vein, some hepatocyte appeared karyolysis and others necrosis (Fig. 3E). But rats treated with tamoxifen after administration of moringa extract (groupV) didn't show any improvement in pathological changes of liver sections showed fibrosis, dilated bile duct and blood sinusoid; degeneration in the form pyknosis, karyolysis, and vacuolar degeneration (Fig. 3F).

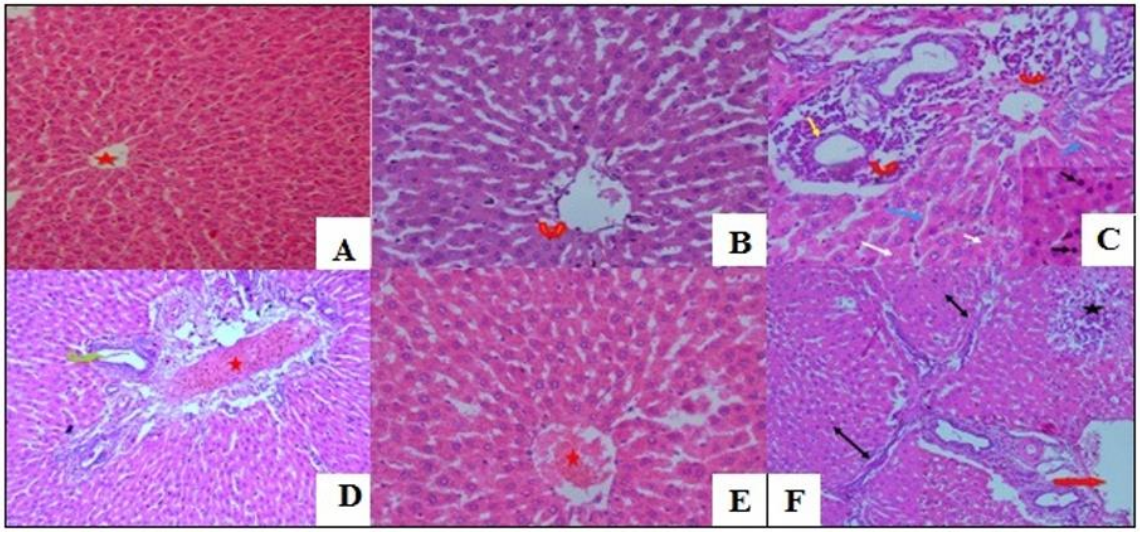


Fig. 3 A; Light micrograph showing liver sections of normal rat showed normal histological structure of hepatic lobules and central vein (star) (H&E100). **B**; Liver sections of the rats treated with moringa aqueous extract only showed liver tissue more or less appeared normal (H&E200). **C**; The microscopic examination of liver sections of rats treated with Tamoxifen only signs of degeneration in the form pyknosis (black arrow), karyolysis (white arrow) and cellular infiltration around bile duct (red curved arrow) (H&E400). **D**; A photomicrograph of the liver of Tamoxifen treated rats showing dilated and congested portal vessel (star) (H&E100). **E**; Photomicrograph of a cross sections in the liver of rat treated with tamoxifen along with moringa extract at the same time showing congestion of central vein (star) (H&E200). **F;** Section of the liver of tamoxifen after administration of moringa extract fibrosis (black arrow), dilated bile vessel (red arrow), aggregation of cellular infiltration (star) (H&E100).

DISCUSSION

Tamoxifen (TMX), a selective estrogen receptor modulator and non-steroidal antiestrogenic drug, is used in the chemotherapy of breast cancer (Kuo *et al.,* 2012; Tsai *et al.,* 2014; Pandey *et al.,* 2016) but many studies reported its adverse effects, such as hot flashes, fatty liver, hepatotoxicity and hepatocarcinomas (Ribeiro *et al.,* 2014; Wickramage *et al.,* 2017). The present study was attempting to investigate the role of moringa oleifera extract, in reducing Tamoxifen side effects.

The present study pointed that intoxication with Tamoxifen® only induced a significant elevation in the activities of ALT; AST and LDH. This finding is agonist many previous studies (El-Beshbishy, 2005; Yang et *al*., 2016 and Wickramage *et al*., 2017). On the other hand, animals' groups treated with tamoxifen along with moringa extract at the same time (group IV) recorded a significant improvement in serum activities of ALT; AST and LDH in comparison to the animals' group treated with tamoxifen alone more than group V this finding is confirmed with that of Okwari *et al*. (2013); Sheikh *et al*. (2014) and Bahr&Farouk (2016).

The obtained data illustrated that tamoxifen alone induced a significant increase in hepatic and renal levels of oxidative markers (MDA and NO) matched with a significant reduction anti-oxidative marker (TAC and GSH) and ATPase activity as compared to the control group. These findings were concomitant with the reports of Nazarewicz *et al*. (2007); Tabassum *et al.* (2007) and Kumarappan *et al.* (2011).

Aminstration of moringa extract at the same time with tamoxifen (groupIV) recorded a significant improvement in hepatic and renal levels MDA, NO, ATPase, TAC and GSHin compare to the animals' group intoxicated with tamoxifen alone (group III) more than group V. This finding is confirmed with that of Saalu *et al.* (2012); Waterman *et al*. (2014) and Bibi *et al*. (2016).

Animals intoxicated with Tamoxifen only revealed a significant reduction in serum levels of total proteins and albumin compared to normal animals; this result reflecting that Tamoxifen manifested a severe inhibition in the hepatic protein synthetic capacity and overall liver damage, and confirm the depth and intensity of liver necrosis inconsistently with the report of Yuvaraj *et al.* (2007) and Kumarappan *et al.* (2011); while treatment with Moringa aqueous extract (group IV&V) attenuated the level of proteins as compared to those intoxicated with Tamoxifen. This finding agonist that of Ghasi *et al*. (2000).

The present study showed that rats treated with Tamoxifen alone showed a disturbance in lipid profile pointed with a significant elevation in the serum level of total cholesterol, triglycerides and LDL-c associated with a decrease in the serum level of HDL-c. These results agree with Yuvaraj *et al.* (2007) while administration of Tamoxifen and aqueous extract of *Moringa oleifera* (group IV) showed a marked improvement in serum lipograme in comparison to those intoxicated with Tamoxifen only. This finding is in accordance with many reports (Ghasi *et al*., 2000; Okwari*et al*., 2013 and Sheikh *et al*., 2014).

On the other hand, animals intoxicated with Tamoxifen revealed a significant increase in serum levels of creatinine and urea as compared with control group with improvement in their levels with administration of aqueous extract of Moringa, confirming the nephrotoxicity effect of Tamoxifen this agonist with Sato *et al*. (2004) and Tabassum *et al*. (2007).

Rats treated only with Tamoxifen (group III) revealed a significant increase level of TNF-α, IL1β and IL10 this result is conflicting that of Zuhair and ALamri

(2011). The animals treated with Tamoxifen and aqueous extract of *Moringa oleifera* showed a significant improvement in serum TNF-α, IL1β and IL10 levels in group IV than group V. These results are in accordance with Panda *et al*. (2013) and Waterman, *et al*. (2014).

Liver sections of rats treated with Tamoxifen only showed signs of degeneration in the form of pyknosis,karyolysis, and cellular infiltration around the bile duct. Also, dilated blood sinusoid, congested portal vessel, dilated bile duct were observed. Additionally, a marked degree of fibrosis, hypertrophy of kupher cells and vacuolar degeneration were noticed. These results are in agreement with Kumarappan *et al*. (2011). Moreover, the section of the livers of rats treated with Moringa extract with Tamoxifen (group IV) exhibited remarkable preservation in the liver histological profiles comparable to the animals' group intoxicated with Tamoxifen alone. This result is in accordance with Saalu*et al*. (2012); Yassar and Tohamy (2014); Bahr and Farouk (2016).

In conclusion from the present investigation offers strong evidence for the hepatoprotective, antifibrotic, anti-inflammatory and anti-oxidative efficiencies of the aqueous extract of *M. oleifera* leaves against tamoxifen hepatotoxicity in male rats.

Abbreviation

Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Lactate dehydrogenase (LDH); Tumor necrosis factor alpha (TNF-α); Interleukin one beta (IL-1β); Interleukin ten(IL-10); Malondialdhyde (MDA); Nitric Oxide (NO); Total antioxidant capacity(TAC);Triglyceride (TG); High density lipoprotein (HDL); Low density lipoprotein (LDL); Reduced glutathione (GSH).

REFERENCES

Ahotupa, M.; Hirsimaki, P.; Parssinen, R.; Mantyla, E. (1994). Alterations of drug metabolizing and antioxidant enzyme activities during tamoxifen-induced hepatocarcinogenesis in rats. *Carcinogenesis*, 15:863-868.

Allen. L. C. Creatinine determination. Clinical Chemistry 1982; 28: 555.

Allan, T.M.; Tyrrelland, P.J.; Rothwell, N.J. (2005). Interlukin1and neuronal injury. *Natural Reviews Immunology*, (5) :629-40.

Anderson, W.F.; Chatterjee, N.; Ershler, W.B.; Brawley, O.W. (2002).Estrogen Receptor Breast Cancer Phenotypes in the Surveillance, Epidemiology, and End Results Database. *Breast Cancer Research and Treatment*, 76(1): 27–36.

Bahr, H.I. and Farouk, S.M. (2016). Comparative Ameliorative Effect of Basil Oil and Moringaoleifera on Lornoxicam- Mediated Histological and Biochemical Alterations in AlbinoRat Liver. *Veterinary Science & Technology*, 7(2);1-11

Berth, M. and Delanghe, J. (2004). Protein precipitation as a possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2 case reports and a review of literature. *Acta Clinica Belgica*, 59:263-267.

Berkovich, L.; Earon, G.; Ron, I.; Rimmon, A.; Vexler, A.; Lev-Ari, S.(2013).Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappa B and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *Complementary and Alternative Medicine*, 13: 212–219.

Berth, M. and Delanghe, J. (2004). Protein precipitation as a possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2 case reports and a review of literature. *Acta*

Clinica Belgica,59:263.

Beutler, E.; Duron, O.; Kelly, B.M. (1963). Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine 61: 882–890.

Bhutada, P.R.; Jadhav, A.J.; Pinjari, D.V.; Nemade, P.R.; Jain, R.D. (2016). Solvent assisted extraction of oil from Moringa oleifera Lam. Seeds. *Industrial Crops and Products,* 82:74–80.

Bibi, A.; Ullah, F.; Mehmood, S.; Bibi, K.; Khan, S.U. (2016). Moringa oleifera Lam. leaf extract as bioregulator for improving growth of maize under mercuric chloride stress. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science,* 66(6) 469-475.

Brouckaert,P. ; Libert ,C. ;Everaerdt ,B.; Takahashi, N.; Cauwels ,A.; Fiers,W. (1993).Tumor necrosis factor, its receptors and the connection with interleukin1and interleukin 6. *Immunobiology*,187(3-5):317-29.

Caballero, E.; Gerez, E.; Oliveri, L.; Falcoff, N.; Batlle, A.: Vazquez, E.(2001).On the promoting action of tamoxifen in a model of hepato-carcinogenesis induced by p-dimethylamino azobenzene in CFI mice. *International Journal of Biochemistry & Cell Biology,* 33:681.

Draper, H.H. and Hadley, A. (1990). Malondialdehyde determination as index of lipid peroxidation. In: Method in Enzymology, Packer, L. and Glazer, A.N. (Eds.), Academic press, San Diego, New York, London, Tokyo, pp.421–431.

Drury, R.B. and wallington, E.A. (1980).carleton's Histological Technique Oxford University press, pp. 520

Eisenwiener,H.G.(1976).Kinetic determination of urea with the LKB system. Journal of clinical chemistry and clinical biochemistry (14) 261-264.

El-Beshbishy, H.A. (2005). Hepatoprotective Effect of Green Tea (Camellia sinensis) Extract against Tamoxifen-induced Liver Injury in Rats. *Journal of Biochemistry and Molecular Biology,* 38:563-570.

Galeone, C.; Pelucchi, C.; Levi, F.; Negri, E.; Franceschi, S.; Talamini, R.; Giacosa, A.; La Vecchia, C. (2006). Onion and garlic use and human cancer. *American Journal of Clinical Nutrition*, 84(5): 1027-1032.

Ghasi, S.; Nwobodo, E.; Ofili, J.O. (2000). Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high-fat diet fed wistar rats. *Journal of Ethnopharmacology,* 69:21–25.

Deroo, P.; Wincke, H.V.; Min, M.; Waters, L.B.F.M.; Verhoelst, T.; Jaffe, W.; Morel, S.; Paresce, F.; Richichi, A.; Stee, P.; Wittkowski, M. (2006). Resolving the compact dusty discs around binary post-AGB stars using N-band interferometry. *Astronomy& Astrophysics*, 450: 181–192.

Desta, Z.; Ward, B.A.; Soukhova, N.V.; Flockhart, D.A. (2004). Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450system in vitro: prominent roles for CYP3A and CYP2D6. *Journal of Pharmacology and Experimental Therapeutics,* 310: 1062-1075.

Jaiswal, D.; Rai, P.K.; Kumar, A.; Mehta, S.; Watal, G. (2009). Effect of moringaoleifera lam. Leaves aqueous extract therapy on hyperglycemic rats. *Journal of Ethnopharmacology*,123:392-396.

Johnson, M.D.; Zuo, H.; Lee, K.H.; Trebley, J.P.; Rae, J.M.; Weatherman, R.V.et al.(2004).

Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Research. Treatment,* 85:151-159.

Johnston, S.R.D. (2005). Selective estrogen receptor modulators and down regulators for breast cancer — Have they lost their way. *Breast Cancer Research*, 7:119–130.

José M , Chávez, E and Jiménez A. Effect of Moringa oleifera seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen, *Journal of Applied Animal Research,* 2020 ;48(1);114-120.

Karthivashan, G.; Umar Kura, A.; Arulselvan, P.; Norhaszalina Md. Isa, N. and Fakurazi, S. (2016). The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. Peer Journal. ; 4: e2127.

Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology,* 54: 356-361.

Kumarappan, C.; Vijayakumar, M.;Thilagam, E. ; Balamurugan, M.; Thiagarajan, M. ; Senthil, S. ; Das, S.C. ; Mandal, S.C.(2011).Protective and curative effects of polyphenolic extracts from Ichnocarpusfrutescense leaves on experimental hepatotoxicity by carbon tretrachloride and Tamoxifen. *Analysis of Heptology*,10 (1): 63-72.

Kuo, J.R.; Wang, C.C.; Huang, S.K.; Wang, S.J.; (2012). Tamoxifen depresses glutamate release through inhibition of voltage-dependent Ca2+ entry and protein kinase Cα in rat cerebral cortex nerve terminals. *Neurochemistry International,* 60:105–114.

McDonnell, D.P. (1999). The molecular pharmacology of SERMs. *Trends in Endocrinology & Metabolism,10*:301–311.

Montgomery, H.A.C. and Dymock, J.F. (1961). The determination of nitrite in water.*Analyst* , 86: 414-416.

Naito, H.K. (2003). Coronary Artery Disease and Disorders of lipid Metabolism Clinical Chemistery in: Theory Analysis Correlation 4thEd., Kaplan, L.A., Pesce , A.J., Kazmierczak S.C. (Mosby , Inc. eds . St Louis USA), pp 603.

Nazarewicz, R.R.; Zenebe, W.J.; Parihar, A.; Larson, S.K.; Alidema, E.; Choi, J. and PedramGhafourifar, P. (2007). Tamoxifen Induces Oxidative Stress and Mitochondrial Apoptosisvia Stimulating Mitochondrial Nitric Oxide Synthase. *Cancer Research,* 67: (3)1282-1290.

Okwari, O.O.; Dasofunjo, K.; Asuk, A.A.; Alagwu, E.A.; Mokwe, C.M. (2013). Anti-Hypercholesterolemic and Hepatoprotective effect of Aqueous Leaf Extract of Moringa oleifera in Rats fed with Thermoxidized Palm Oil Diet. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS),* 8(2): 57-62.

Paget, G.E. and Thomson, R. (1979). Standard operating procedures in toxicology. MTP Press limited, Lancaster, England.

pala, S.; Atilgan, R.; Ozkan, Z.S.; Kavak,S.B. ; Ilhan, N.;Akpolat, N ; sapmaz, E.(2015) . Effect of varying doses of tamoxifen on ovarian histopathology, Serum VEGF and endothelin l levels in ovarian hyper stimulation syndrome an experimental study. Journal of drug design, *Development and Therapy*,9: 1761-

1766.

Palafox, J.O.; Navarrete, A.; Sacramento-Rivero, J.C.; Rubio-Atoche, C.; Escoffie, P.A.; Rocha-Uribe, J.A. (2012). Extraction and characterization of oil from Moringa oleifera using supercritical CO2and traditional solvents. *American Journal of Analytical Chemistry*, 3: 946–949.

Panda, S.; Kar, A.; Sharma, P.; Sharma, A.(2013).Cardioprotective potential of N,a-L-rhamnopyranosylvincosamide,anindole alkaloid, isolated from the leaves of Moringa oleifera in isoproterenol induced cardiotoxic rats: In vivo and in vitro studies. *Bioorganic & Medicinal Chemistry Letters*, 23 :959–962.

Pandey, D.; Banerjee, S.; Basu, M.; Mishra, N. (2016). Memory enhancement by Tamoxifen on amyloidosis mouse model. *Hormones and Behavior*, 79 :70–73.

Ribeiro, M.P.C.; Santos, A.E.; Custódio, J.B.A. (2014). Mitochondria: The gateway for tamoxifen-induced liver injury. *Toxicology*, 323: 10-18.

Saladores, P.; Mürdter, T.; Eccles, D.; Chowbay, B.; Zgheib, N.K.; Winter, S.; Ganchev, B.; Eccles, B.; Gerty, S.; Tfayli, A.; Lim, J.S.L.; Yap, Y.S.; Ng, R.C.H.; Wong, N.S.; Dent, R.; Habbal, M.Z.; Schaeffeler, E.; Eichelbaum, M.; Schroth, W.; Schwab, M.; Brauch, H. (2015). Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. *The Pharmacogenomics Journal*, 15: 84–94.

Saalu, L.C.; Ogunlade, B.; Ajayi, G.O.; Oyewopo, A.O.; Akunna, G.G.; Ogunmodede, O.S. (2012). The hepatoprotective potentials of Moringa oleifera leaf extract on alcohol-induced hepato-toxicity in wistar rat. *American journal biotechnology and molecular sciences*, 2(1): 6-14.

Sato, T.; Tanaka,K.; Ohnishi,Y.; Teramoto, T.; Irifune,M. ;Nishikawa, T.(2004).Effects of steroid hormones on (Na+, K+)-ATPase activity inhibition- induced amnesia on the step-through passive avoidance task in gonadectomized mice. *Pharmacological Research*, 49(2)151–159.

Schumann, G.; Klauke, R.(2003).New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects.ClinChim Acta.,327(1-2):69-79.

Siegel, R.; Naishadham, D.; Jemal, A. (2012). Cancer Statistics.CA: *CANCER Journal of Clinicians*, 62:10–29.

Shoda, S.; Kato, M.; Harada, R.; Fujisato, T.; Okuhira, K.; Demizu, Y.; Inoue, H.; Naito, M.; Kurihara, M. (2014). Design and synthesis of tamoxifen derivatives as a selective estrogen receptor down-regulator. *Bioorganic & Medicinal Chemistry Letters*, 24: 87–89.

Sheikh,A.; Yeasmin,F.; Agarwal,S.; Rahman,M.; Islam,K.; Hossain,E.; Hossain, S.; Karim, M.R.; Nikkon,F.; Saud, Z.A.; Hossain, K.(2014). Protective effects of Moringa oleifera Lam. leaves against arsenic-induced toxicity in mice. *Asian Pacific Journal Trop Biomedicine*,4(Suppl 1): S353-S358.

Tabassum, H.; Parvez, S.; Rehman, H.; Banerjee, B.D.; Siemen, D.; Raisuddin, S. (2007). Nephrotoxicity and its prevention by taurine in tamoxifen induced

oxidative stress in mice. *Human & Experimental Toxicology*, 26: 509–518.

Tietz, N. W. (1983). A Reference Method for the Measurement of ALP Activity in Human Serum. Study group on ALP. *Journal of Clinical Chemistry*, 29: 751-754.

Tomao, F.; Colombo, N.; Panici, B. (2015). Is endometrial cancer risk reduced by oral bisphosphonate use? *Journal of Clinical Oncology*, 33:3670-3671.

Tsai, Y.T.; Wang, C.C.; Leung, P.O.; Lin, K.C.; Chio, C.C.; Hu, C.Y.; Kuo, J.R. (2014). Extracellular signal–regulated kinase 1/2 is involved in a tamoxifenneuroprotective effect in a lateral fluid percussion injury rat model. *Journal of Surgical Research,*189: 106–116.

Tsakiris, S.; Schulpis, K.H.; Marinous, K. and Behrakis, P. (2004). Protective effect of L-cysteine and glutathione on the modulated sukling rat brain Na+/K+-ATPase and Mg2+-ATPase activities induced by the in vitro gulactosaemia. *Pharmacology Research*, 49: 475-479.

Upadhyay, P.; Yadav, M.K.; Mishra, S.; Sharma, P.; Purohit, S. (2015). Moringa oleifera: a review of the medical evidence for its nutritional and pharmacological properties. *International Journal of Research Pharmaceutical*, 5, 12–16.

Vancutsem, P.M.; Lazarus, P.; Williams, G.M. (1994). Frequent and specific mutations of the rat p53 gene in hepatocarcinomas induced by tamoxifen. Cancer Research.54: 3864-3867.

Waterman, C.; Cheng a, D.M.; Rojas-Silva, P.; Poulev, A.; Dreifus, J.; Lila, M.A.; Raskin, I.(2014). Stable, water extractable isothiocyanates from Moringa oleifera leaves attenuate inflammation in vitro. *Phytochemistry*, 103 :114–122.

Wickramage, I.; Tennekoon, K.H.; Ariyaratne, M.A.; Hewage, A.S.; Sundralingam,T.(2017). CYP2D6 polymorphisms may predict occurrence of adverse effects to tamoxifen: a preliminary retrospective study. *Breast Cancer - Targets and Therapy,9*:111–120.

Yang, G.; Nowsheen, S.; Aziz, K.; Alexandros G. Georgakilas, A.G. (2013). Toxicity and adverse effects of Tamoxifen and other anti-estrogen drugs. *Pharmacology & Therapeutics*, 139: 392–404.

Yang,Y.J.; Kim,K.M.; An,J.H.; Lee,D.B.; Shim,J.H.; Lim, Y.S.; Lee, H.C.; Lee,Y.S.; Ahn, J.H.; Jung, K.H.; Kim, S.B.(2016).Clinical significance of fatty liver disease induced by tamoxifen and toremifene in breast cancer patients. *The Breast*, 28:67-72.

Yassar, H.D. and Tohamy,A.F.(2014). Extract of Moringa oleifera leaves ameliorates streptozotocin-induced Diabetes mellitus in adult rats. *Acta Histochemica*, 116:844–854.

Yuvaraj , S.; Premkumar,V.G.; Vijayasarathy , K.; Gangadaran , S.G.; Sachdanandam, P.(2007) . Ameliorating effect of coenzyme Q10, riboflavin and niacin in tamoxifen-treated postmenopausal breast cancer patients with special reference to lipids and lipoproteins. *Clinical Biochemistry*, 40: 623–628.

Zuhair, Z. and ALamri, H. (2011). The role of vitamin C in alteration of enzymes responsible of energymetabolism induced by administration of tamoxifen to mouse. *Advances in Biological Chemistry*, 1:15-23.

ARABIC SUMMARY

الدور الفعال لمستخلص المورينجا كمضاد للأكسدة والالتهاب ضد سمية الأدوية الكيميائية لعلاج سرطان الثدى في جرذان التجارب

عبد النبي ابراهيم عيسوي [1] ، هدير محمد بكير [1] ، علا نبيل سيد [2] ،خالد جمال الدين عبدالوهاب [3] ، وشيماء ربيع صابر [1]

1–قسم الكيمياء- كلية العلوم - جامعة الفيوم

2- قسم الكيمياء الحيوية – كلية العلوم – جامعة الفيوم

3–قسم الفسيولوجيا الطبية - المركز القومى للبحوث

سرطان الثدي هو أكثر أنواع السرطان شيوعا يسبب الوفاة عند النساء. تاموكسيفين هو عقار مضاد للإستروجين لمنع سرطان الثدي المسبب لتسمم الكبد. أجريت هذه الدراسة لتوضيح فاعلية مستخلص المورينجا أوليفيرا المائي ضد تأثير عقار تاموكسيفين المسرطنة للكبد في ذكور الجرذان. تم تقسيم 50 من ذكور الجرذان البيضاء بالتساوي إلى خمس مجموعات: المجموعة الأولى وهى المجموعة الضابطة ؛ مجموعة مورينجا أوليفيرا المائية المعالجة (المجموعة الثانية) بجرعة (300 مجم / كجم / يوم لمدة 6 أسابيع) ؛ المجموعة (الثالثة) تلقت عقار تاموكسيفين (3 مجم / كجم / 3 أيام لمدة 6 أسابيع) ؛ تلقت الجرذان مستخلص مائي تاموكسيفين ومورينجا أوليفيرا سويًا لمدة 6 أسابيع للمجموعة (المجموعة الرابعة). وجرذان تمت معالجتها مسبقًا بمستخلص المورينجا أوليفيرا المائي ثم تاموكسيفين بنفس الجرعة و الفترة (المجموعة الخامسة). في المجموعة الثالثة كان هناك ارتفاع كبير في أنشطة كلا من الانزيمات الناقله لمجموعه الأمين (الالانين والاسبارتيك) ومستويات علامات الالتهاب والانترلوكينات وكذلك الكوليسترول والدهون بالاضافه الي إرتفاع مستوي اكسيد النيتريك ومستوي الاكسده الفوقيه للدهون (المالونديالدهيد). كان هناك انخفاض معنوي في البروتينات الكلية والألبومين ومضادات الأكسدة مقارنة بمجموعة التحكم. أدى تناول مستخلص المورينجا المائي عن طريق الفم إلى تحسين العلامات الكيميائية الحيوية وتوفير نشاط مضاد للأكسدة والالتهاب في ضد سمية تاموكسيفين في أنسجة الكبد بإرتفاع ملحوظ في البروتينات الكلية والألبومين ومضادات الأكسدة وإنخفاض في أنشطة الانزيمات الناقله لمجموعه الأمين (الالانين والاسبارتيك) وتحسن واضح في مستويات علامات الالتهاب.