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# GRAPE SEED EXTRACTED (VITISVINIFERA) ALLEVIATE HEPATIC TOXICITY INDUCED BY THE ANTI-OESTROGEN TAMOXIFEN IN FEMALE ALBINO RATS

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**Keywords:**

Grape Seed Extract, Tamoxifen, Antioxidant, liver toxicity, flowcytometry, Rat

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**ABSTRACT: Aim:** The present study has been undertaken to investigate the therapeutic effect of GSE (Grape seed extracts) against Tamoxifen (TAM), induced hepatotoxicity in rat. **Methods:** The rats were divided into four groups • Group 1: rats were injected intraperitoneally (i.p.) with saline for seven days. • Group 2: Rats were treated with TAM in a dose of 45 mg/kg b·w/day, i.p., for seven successive days. • Group 3: Rats were administrated orally GSE (100 mg/kg b·w/day) for three weeks. Group 4: rats were injected (i.p.) (45 mg/kg b·w/day) of Tamoxifen for seven days, then treated daily with a single dose of GSE (100 mg/kg b·w/day) for three weeks respectively. **Results:** GSE reduced necrosis in the TAM-treated rat. And significantly increased ($p<0.05$) the levels of MDA and PCC, while the level of GSH was significantly ($P<0.05$) decreased. Treatment with GSE significantly ($P<0.05$) reduced. Tamoxifen significantly decreased ($P<0.05$) the level of NO. GSE significantly increases ($P<0.05$, Table 2) NO level. Flowcytometric analysis in liver cells, significant increases in apoptotic cells treated by TAM and decreases in the group exposed to TAM and treated with GSE. **Conclusion:** This study suggests that GSE possesses anti-oxidant effects against TAM toxicity.

**INTRODUCTION:** Tamoxifen (TAM), a triphenylethylene derivative, is a selective estrogen receptor modulator (SERM) [60] that has become the treatment of choice for women diagnosed with all stages of hormone-responsive breast cancer**.** Breast cancer remains the most common malignancy in women worldwide. Estrogen levels appear to be associated with an increased risk for the development of breast cancer. In 1998, the National Surgical Adjuvant Breast and Bowel Project (NSABP) demonstrated that tamoxifen treatment reduced the incidence of both invasive and non-invasive breast cancer in the population at high risk for disease [64].

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It was revealed that TAM in a high dose is a known liver carcinogen in rats [2, 5] which is due to oxygen radical overproduction, which occurs during TAM metabolism. The hepatotoxicity of TAM to the rat has been demonstrated in numerous studies [62] and malignant transformations in the human endometrium [33, 41]. It was suggested that TAM initially metabolized in the liver with subsequent accumulation of some metabolites such as 4-hydroxytamoxifen, 4 – hydroxy – N - desmethyl tamoxifen, and N-des dimethyl tamoxifen in various tissues [6].

Clinical data suggest that tamoxifen can cause changes in the endometrium ranging from reversible thickening of the lining, via dysplasia and glandular hyperplasia, to the formation of polyps and ultimately full-blown carcinosarcomas [53]. It has been shown by other investigators that tamoxifen can cause or exacerbate hepatic dysfunction [43, 67].

Plant material in the human diet contains a large number of natural compounds, which may be of benefit in protecting the body against the development of neuron-toxicity. One of the first plants with constituents reputed to possess neuro protective property was grape. The Grape-vine (Vitisvinifera), is cultivated today in all temperature regions of the world [32]. Its seeds contain several active components, including flavonoids, polyphenol, an- thocyanins, proanthicyanidins and procyanidines, grape seeds extract (GSE) contain 70% - 95% standardized proanthocyanidins [73].

A variety of naturally-occurring grape seed extracts to have been found to have beneficial effects on health, and these compounds are drawn attention because of their relative safeness and accumulated evi- dence of physiological properties in animals and human [58]. Oil produced from grape seeds is considered a rich source poly phenolic with strong antioxidant activity, chemopreventive, anti-inflammatory, anti-microbial and anti-cancer effects [22, 69].

As a result, research into the toxic effects of tamoxifen continues and the last decade has been particularly fruitful in providing new information on manifold influences of this drug. Although the anti-cancer activity of Grape seed extracts components was recognized thousands of years ago, but proper scientific research with this important traditional medicine is a history of last 2~3 decades. There are not so many research works done with this important traditional medicine and very few reports existed in the scientific database. Therefore, the present study attention is focused on whether the administration of grape seeds extracts to rats could protect and therapeutic against tamoxifen-induced hepatic toxicity.

## MATERIALS AND METHODS:

**Chemicals**: Tamoxifen citrate (TAM), was a kind gift obtained from the medical union pharmaceutical drug company (MUP), Egypt. All the other chemicals were of analytical grade and purchased from Sigma Company, Cairo branch.

**Preparation of grape seed extracts (GSE):** Grapes as large clusters with red berries, were bought from a local supermarket in Egypt (Cairo). Grape seeds were removed from the grapes, air dried for one week. The ethanol extracts was prepared by soaking 100 gm of grape seeds powdered in 300 ml ethanol (95%) shaking (24 h) then covered by a piece of aluminum foil and kept in the refrigerator. The infusion was filtered by a piece of double gauze and the filtrate was centrifuged at 3000 rpm for 10 minutes, then the supernatant (ethanol) was evaporated using a rotatory evaporator apparatus attached to a vacuum pump. The 100 gm of dried grape seeds powder yield 26.7 gm ethanol [30, 45].

**Animals:** Tow-month old (160 - 200 g body weight) female albino rats (*Rattus rattus*) were selected from animal house of National Research Center, Giza, Egypt. The animals were housed under controlled environment conditions (12 h light/dark cycle) at a temperature of 25˚C + 10˚C and humidity of 60% + 5% and fed standard diet and water Ad libitum for the experimental period.

**Experimental protocol:** The rats were randomly divided into four groups of 12 animals each as follows:

- **Group 1:** served as negative control (rats were injected intraperitoneally (i.p.) with ml of normal saline for seven days.
- **Group 2:** (positive control group) TAM-intoxicated rats: Rats were treated with TAM in a dose of 45 mg/kg b·w/day, i.p., for seven successive days [23].
- **Group 3:** GSE rats: Rats were administrated orally GSE (100 mg/kg b·w/day) diluted with saline solution; for three weeks [28, 45].
- **Group 4:** (therapeutic group), rats were injected (i.p.) with a sub-acute dose (45 mg/kg b·w/day) of Tamoxifen for seven days, then treated daily with a single dose of GSE (100 mg/kg b·w/day) for three weeks respectively.

**Examinations**: Rats of each group were sacrificed by cervical dislocation at the end of the experimental periods and decapitation. Liver of each animal was obtained and divided into three samples; one of them was kept in buffered neutral formalin for histological examinations while the

other was kept in liquid nitrogen for flow-cytometrically analysis. Also, the third part of liver tissue stored at -80ºC for proceeding biochemical examination.

**Histopathological studies:** Liver specimens of all groups were collected and fast dissected, and then they were fixed in an aqueous Bouin solution, dehydrated through alcohols, cleared in xylene and embedded in paraffin wax according to the method described by Drury and Wallington [52]. Five-micrometer thickness paraffin sections were prepared and mount on clean slides. For histopathological studies, such as sections were stained with Ehrlich.s hematoxylin and counterstained with eosin.

**Biochemical analysis:** The third part of the collected liver samples were divided into two groups. A and B. Sample of the group (A) were homogenated in iced 70% methanol, centrifuged and supernatant of homogenates tissues were processed for the biochemical analysis included: oxidative stress (MDA and GSH) all were determined by HPLC according to the methods of Karatepe and Liu respectively [34, 42].

The samples of the second group (B) of the liver were weighed and homogenized in 0.1M Potassium Phosphate Buffer (pH 7.4), centrifuged and supernatant of homogenates tissues was processed for the biochemical analysis included: PCC, NO (as total nitrate), all were measured using the spectral-photometric methods of Ellman and Levine respectively [16, 39].

**Flow cytometrically examination:** Flow cytometry analysis was carried out as described previously by I.H. Hirsch [27]. Immediately after samples of liver tissue were removed, they were immersed in cold RPMI liquid. Within 1 hour, each sample was mechanically disaggregated; hand homogenized and filtered through a 50 ìm nylon filter. After washing and centrifugation, the suspension was diluted to a concentration of 2X 106 nuclei per ml, and then divided into two samples; one of them was stained immediately while the other was stained after addition in equal proportions of lymphocytes from healthy donors (internal reference). For each sample minimum of 10,000 nuclei (range 10,000 to 100, 000) was stained in a solution containing 50mg/ml propidium iodide, 2mg/ml ribonuclease and the 1 percent Triton. After repeat filtration with 50 ìm nylon filter, samples were analyzed on flow cytometer equipment. Each DNA histogram was analyzed for peak position and for the percentage of cells in the different histogram regions of different groups. A minimum of 10,000 cells was analyzed by a FACSort (Becton Dickinson, Immuno cytometry Systems, San Jose, CA, USA). The excitation wave length was 488 n mat 150 mW, 10,000 nuclei/ specimen. Histogram analysis of the red fluorescence emitted by the propidium iodide was accomplished manually by setting markers around the haploid (n), diploid (2n), and tetraploid (4n) peaks and calculating the percentage of each ploidy compartment.

**Statistical analysis:** The present data were analyzed by using SPSS11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA). $P<0.05$was considered statistically significant.

**RESULTS:**

**Histopathological results:** The liver sections of the control group exhibited normal architecture where it consists of a roughly hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center (**Fig. 1A**). While the liver of rats treated with Tamoxifen only showed hydropic degeneration, nuclei with variable sizes and dysplastic cells (**Fig. 1B**). Fatty changes, vacuolar degeneration, mitotic figure and fibrosis were seen (**Fig. 1C**). Dilation, congestion of the blood sinusoid and peripheral chromatin clumping were also observed (**Fig. 1B, C**, and **D**).

Light microscopic examinations demonstrated that liver tissue of the animals administered grape seed extract (GSE) had a view similar to normal **(Fig. 1E**).

Concerning rats treated tamoxifen for one week then followed by GSE for three successive weeks as a therapeutic group, showed some protective effects as compared to the group of rats subjected to Tamoxifen only. Examination of liver sections revealed marked restoration of the hepatic configuration.

Most nuclei exhibited normal shape, being rounded and centrally located except for few pyknotic ones. No inflammatory changes were observed (**Fig. 1F**).

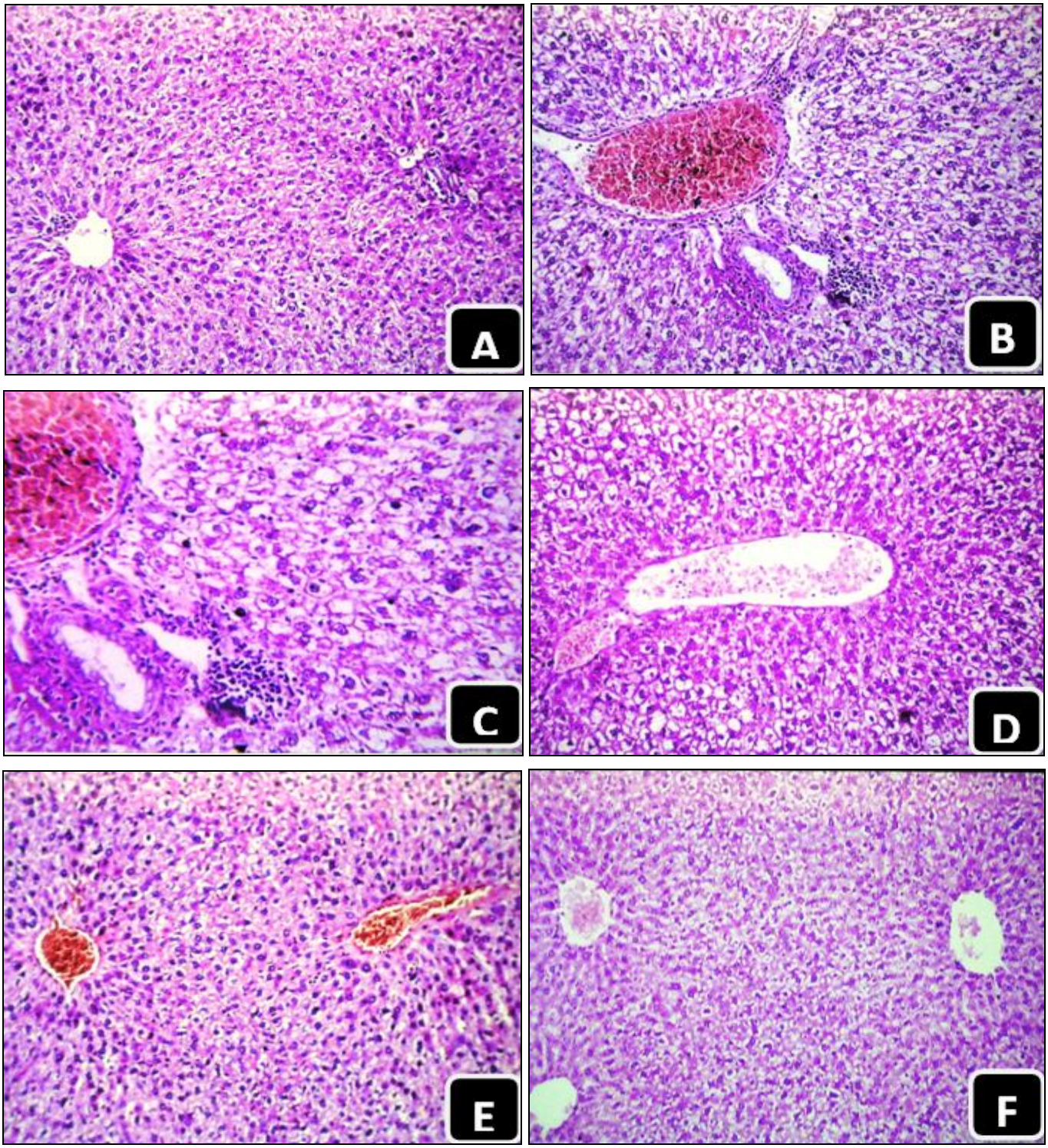


**FIG. 1: A) GROUP 1: NEGATIVE CONTROL LIVER, THERE WAS NO HISTOPATHOLOGICAL ALTERATION OBSERVED AND THE NORMAL HISTOLOGICAL STRUCTURE OF THE CENTRAL VEIN, PORTAL AREA AND SURROUNDING HEPATOCYTES WERE RECORDED (H&E 100). B, C AND D) GROUP 2: POSITIVE CONTROL OF LIVER TREATED WITH TAM, SEVER DILATATION AND CONGESTION WERE NOTICED IN THE CENTRAL AND PORTAL VEIN ASSOCIATED WITH VACUOLAR DEGENERATION IN THE HEPATOCYTES AND DILATATION IN THE BILE DUCT WITH PERIDUCTAL FIBROSIS AND INFLAMMATORY CELLS INFILTRATION IN THE PORTAL AREA (H&E. 200, 250, 200 RESPECTIVELY). E) GROUP 3: LIVER TREATED WITH NATURAL PRODUCT GSE, THERE IS NO HISTOPATHOLOGICAL ALTERATION (H&E 100). F) GROUP 4: THE RATS TREATED WITH TAM FOR ONE WEEK THEN FOLLOWED BY GSE FOR ANOTHER 3 SUCCESSIVE WEEKS, THERE IS MILD HEPATIC ALTERATION AND APOPTOTIC NUCLEI (H&E 100).**

**Biochemical assays:** MDA, PCC, and GSH in liver tissues: The result of this study showed that Tamoxifen treated rats significantly increased ($p<0.05$) the levels of MDA and PCC (Table 1) while the level of GSH was significantly ($P<0.05$) decreased (**Table 2**). Treatment with GSE accompanied with significantly ($P<0.05$) reduced the ROS generation (by decreasing levels of MDA & PCC and increasing the level of GSH) induced by Tamoxifen treatment.

As shown in **Table 2** the i.p injection of Tamoxifen significantly decreased ($P<0.05$) the level of NO as compared with the normal control group. Treatment with GSE significantly increases ($P<0.05$, **Table 2**) NO level.

**Flowcytometric analysis (DNA content in liver cell measurement):** In liver cells, as most dividing cells, the DNA content immediately after division, represents the diploid chromosomal complement (2N); this increases during the DNA synthesis phase (S). The The flowcytometer technique measures the amount of DNA per cell by quantization the intensity of the fluorescence emitted by a DNA-bound dyes flow past a high-intensity laser beam. Parameters of cell cycle analysis of liver samples in all groups using flowcytometry related to histopathologic diagnosis are shown in **Table 3** and **Fig. 2**.

Rats treated with GSE for 3 weeks after injection by Tamoxifen for 7 days (group 4) are more or less similar to GSE treated group (group 3), and both groups are significantly increased than the control group (group 1) in both S phases and G2/M phases (9.50 and 1.37 respectively), as well as in G0/1 phase (16.90) of group 1. When compared to Tamoxifen treated group (group 2) there is a significant increase in groups 3 and 4 at S phase and significantly decreased at G1 and G2 phases. However, the value of apoptosis is significant decreases in Tamoxifen, and GSE treated group (group 4) (12.70) when compared with Tamoxifen treated group (group 2) which shows a significant increase value (19.26). (**Table 3**) and (**Fig. 2**).

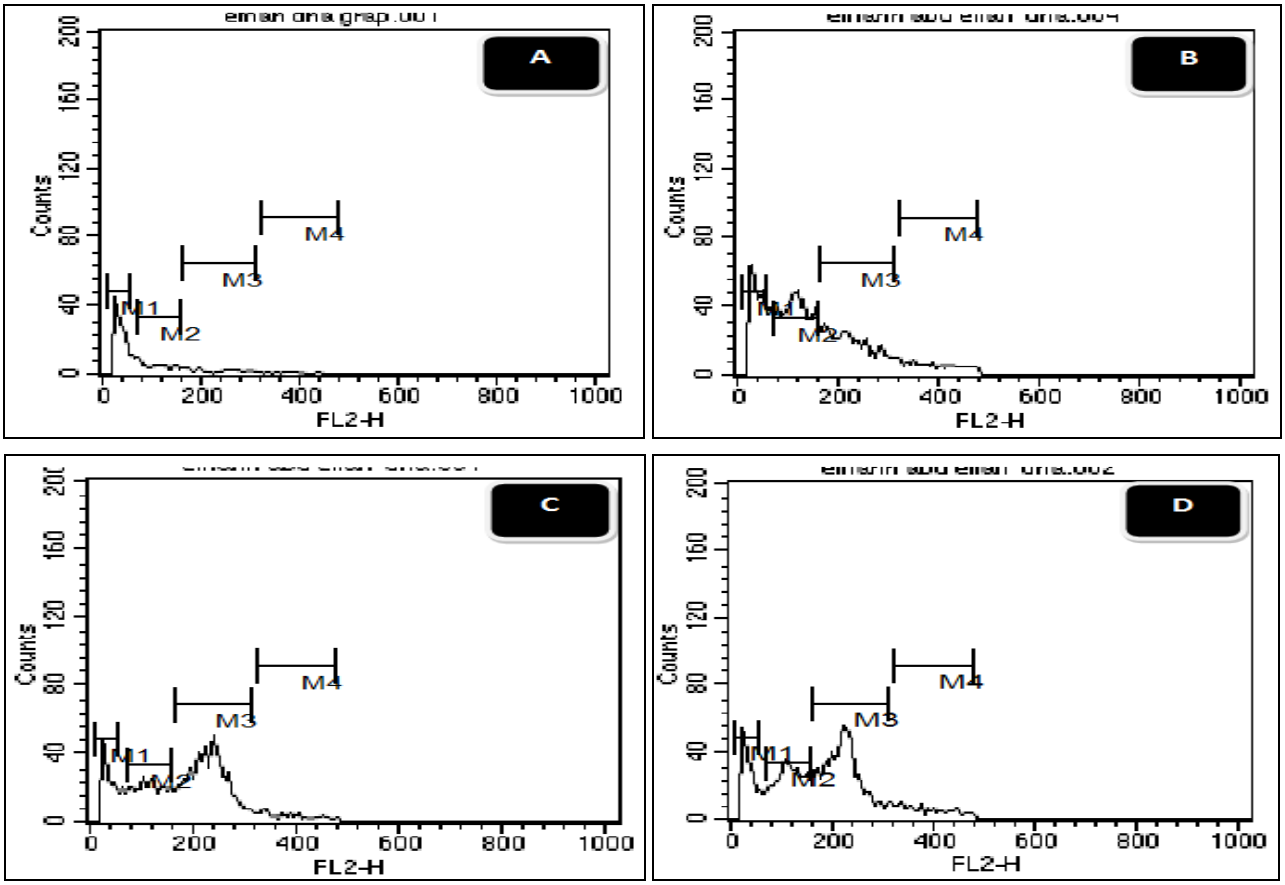


**FIG. 2: FLOWCYTOMETRICAL DIAGRAMS OF DIFFERENT GROUPS, A) NEGATIVE CONTROL GR1, B) POSITIVE CONTROL (TAMOXIFEN TREATED GROUP) GR2, C) TREATED GROUP (GRAPE SEED EXTRACT GROUP) GR3, D) THERAPEUTIC GROUP (TAMOXIFEN THEN GRAPE SEED EXTRACT) GR4. WHERE, M1= APOPTOSIS, M2= G0//1, M3= S%, M4= G2/M**

**TABLE 1: THE EFFECT OF GSE ON THE LEVELS OF MALONDIALDEHYDE (MDA) (µmole/ g WET TISSUE), PROTEIN CARBONYL CONTENT (PCC) (mM/ g WET TISSUE) IN LIVER TISSUE OF FEMALE ALBINO RATS TREATED WITH TAMOXIFEN.**

| Animal Groups | MDA | PCC |
|---|---|---|
| $GP_I$ | 4.582 ± 0.073 | 269.14 ± 9.48 |
| $GP_{II}$ | 6.728 ± 0.319 * | 367.68 ± 6.48 * |
| $GP_{III}$ | 4.601 ± 0.131 | 257.55 ± 10.45 |
| $GP_{IV}$ | 4.710 ± 0.169 # | 293.36 ± 9.19 # |

**TABLE 2: THE EFFECT OF GSE ON THE LEVELS OF NITRIC OXIDE (NO) (µmole/L) AND REDUCED GLUTATHIONE (GSH) (µg/ g WET TISSUE) IN LIVER TISSUE OF FEMALE ALBINO RATS TREATED WITH TAMOXIFEN.**

| Animal Groups | NO | GSH |
|---|---|---|
| $GP_I$ | 119.97 ± 2.00 | 0.833 ± 0.022 |
| $GP_{II}$ | 102.78 ± 1.97 * | 0.722 ± 0.010 * |
| $GP_{III}$ | 118.02 ± 1.78 | 0.772 ± 0.011 |
| $GP_{IV}$ | 113.84 ± 2.05 # | 0.751 ± 0.029 |

**TABLE 3: THE APOPTOSIS PERCENTAGE AND THE CELL CYCLE PHASES IN DIFFERENT EXPERIMENTAL GROUPS (FLOWCYTOMETRIC STUDIES ON LIVER TISSUE RATS TREATED WITH TAMOXIFEN AND GRAPE SEED EXTRACT).**

| Marker | Control Gr1 | Tamoxifen Gr 2 | Grape seed extracts Gr3 | Therapeutic Gr 4 (tamoxifen then grape seed extract) |
|---|---|---|---|---|
| M1= Apoptosis% | 19.26 | 61.03 | 12.65 | 12.70 |
| M2= G0/1 phase % | 36.94 | 16.90 | 24.40 | 26.12 |
| M3= S phase % | 27.53 | 9.50 | 53.51 | 49.76 |
| M4= G2/M phase % | 7.08 | 1.37 | 3.43 | 6.43 |

**DISCUSSION**: Tamoxifen is a triphenyl ethylene derivative commonly used in the treatment of breast cancer [33, 44]. Tamoxifen is known to have varied biological effects ranging from complete estrogen antagonism to pure estrogen agonism depending upon its concentrations, the sex of animals and target organ [70]. In humans and rats, Tamoxifen is predominantly antiestrogenic with residual estrogenic activities [19]. It was obvious that tamoxifen in toxic doses led to oxidative liver damage [23], as it has been elucidated to be a hepatocarcinogen in rats [15, 35]. It may be more toxic to the liver because it has much higher affinity for hepatic tissue than for any other tissues [10]. It was found to produce five DNA adducts in rat liver that appeared to be responsible for carcinogenesis [24]. This study was conducted in order to investigate the role of GSE in alleviating the oxidative stress status produced after TAM-intoxication in female rats that resulted in liver injury.

The results obtained from this study demonstrated that TAM administration resulted in abnormal histopathological changes in the form of dilatation of central veins, vacuolated cytoplasm of hepatocytes these results were in agreement with the results reported that TAM administration resulted in significant elevations of liver enzyme's activities that were confirmed by abnormal histopathological changes that lead to parenchymal cell apoptosis, inflammation and liver cell necrosis [14]. The present study showed that administration of TAM caused prominent fragmented nuclei these results were in agreement with those noticed that the treatment of rats with TAM at dose level of 45 mg/kg bw for two weeks caused vacuolar degeneration in the liver of rats [25, 36].

We also showed that TAM caused dilated blood sinusoids and containing many Kupffer cells, and the cytoplasm had been small fragmented pycnotic cellular nuclei these results were in agreement with the result found that the treatment of rabbit with TAM at a dose level of 14 mg/kg bw daily for 60 days induced histopathological changes in the testis in the form of vacuolar degeneration of spermatogenic cells, atrophied and collapsed seminiferous tubules with asospermia [3].

TAM or triphenylene compounds undergo metabolic activation reactions, such as 4-hydroxylation, 3 hydroxylation, α-hydroxylation, N-demethylation, etc., in animal tissues [46, 62], which raised the production of reactive oxygen species (ROS) [53]. Great attention now focused on the clinical usage of this drug because it's several diverse effects, mainly idiosyncratic hepatotoxicity [7]. Our results show that TAM increase lipid peroxidation with a high level of malondialdehyde (MDA) as a main product of lipid breakdown MDA is the major aldehydes formed after the breakdown of lipid hydroperoxides.

According to Donaldson reported that the determination of MDA levels is still the most commonly applied assay for lipid peroxidation in

biomedical sciences since MDA is the major aldehydes formed after the breakdown of lipid hydroperoxides [12]. Öktem mentioned that tamoxifen toxicity leading to free-radical damage by two pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of Lipid peroxidation, and (2) the direct depletion of antioxidant reserves [49].

Additionally, in the current study, the observation of PCC was significantly higher in Tamoxifen-treated rats than normal control rats; these results are in accordance with Tham, C. S., who demonstrated that protein modifications elicited by direct oxidative attack and increasing in ROS, thus leading to the formation of protein carbonyl derivatives because PCC is generated by the actions of reactive oxygen species (ROS) mediated carboxylation of protein. In the same line **[66]**, El-Masry, added that reactive oxygen species could also attack directly at polypeptides of proteins and result in increasing carbonyl groups, the levels of which were described as drug-catalyzed oxidation of proteins and were often taken as presumptive evidence of oxidative modification in protein [17].

On another side, as shown in this study, Tamoxifen caused a dramatic increase in MDA level, which accompanied by a decrease in GSH levels in liver tissues. Where Tamoxifen decreased the levels of liver GSH through its strong affinity for the (-SH) group (binds exclusively to the -SH group of GSH). Previously, the present data showed that high MDA, PCC, and low GSH in the Tamoxifen group, indicating heavy oxidative damage and low antioxidant levels [31, 66].

The analysis of data showed that the activity in the liver tissue of Tamoxifen-exposed groups was lower than that of the control one**.** Nitric oxide is a highly reactive oxidant produced by the liver parenchymal and non-parenchymal cells from L-arginine via nitric oxide synthase [11, 40]. Nava-Ruíz added that NO can produce in the liver tissue by the stoichiometric conversion of L-arginine to L-citrulline via three oxide synthase (NOS) isoforms: constitutive $Ca^{2+}$/Calmodulin - dependent endothelial NOS (eNOS) and neuronal NOS (nNOS), and the inducible $Ca^{2+}$ independent NOS (iNOS) [48]. Hussein proposed that serum NO level was decreased in the Tamoxifen-treated group due to free radical formation leading to increasing the presence of superoxide anion and raising the probabilities of an interaction between NO and ROS to produce a peroxynitrite (highly deleterious molecule) [26].

The recorded results explained certain consistencies regarding the role of flow cytometry in the assessment of large hepatic injury induced by Tamoxifen. The distribution for the control group, there was no significant of aneuploidy in G2. Sutherland described tamoxifen as a cell cycle phase. Specific growth inhibitory agent, which blocks cells in the GO/Gl phase of the cell cycle [63], and Osborne suggested that tamoxifen inhibited cell proliferation by invoking a transition delay or block in the early-to-mid-G1 phase of the cell cycle [50]. We find that tamoxifen treatment reduces the growth fraction considerably and both cells with a G1 amount of DNA and cells with a G2 amount of DNA are growth arrested.

The results obtained. Showing a significant increase in the percent of apoptotic cells in Tamoxifen-induced hepatocellular toxicity, and this group was reversed with GSE treatment. In this respect, M.S. Babu mentioned that, administration of GSE significantly inhibited the development of colonic aberrant crypt's carcinomas and liver carcinoma [47]. Since GSE polyphenolic partially protect DNA from OH radical-induced strand breaks and base damage through fast chemical repair [54]. In addition, polyphenol provide cytoprotective and DNA protection against oxidative stress may involve the following suggested mechanism, that the hydroxyl grouped in the aromatic B ring of polyphenol are considered important in scavenging free radicals [65], where the additional hydroxyl groups in polyphenol make it the most effective in reactive oxygen species (ROS) scavenging.

Moreover, GSE significantly reduced liver MDA and PCC contents and increased liver GSH and NO contents in a dose-dependent manner [57]. GSE contains polyphenolic compounds such as procyanidins and proanthocyanidins that have a powerful free radical scavenging effect [4, 17]. The previous study demonstrated that GSE prevents

hepatic fibrosis and dysfunction caused by the chronic arsenic administration in rats [51].

Grape seeds extract (GSE) contains polyphenol including proanthocyanidins and procyanidins that showed antioxidant and free radical scavengers, being more effective than either ascorbic acid or vitamin E [8, 72]. Their effects include the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals. Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals [69]. Also blocks cell death signaling [56].

Many studies have provided evidence that proanthocyanidin has potent radical scavenging ability, antioxidant properties and significant neuro protective as well as a cardiovascular protective effect [17, 21]. Procyanidins are natural botanic polyphenol extracted from grape seeds, with bioactivities such as antioxidation, free radical elimination, and cell proliferation stimulation [8]. Recently, there has been great evidence that GSE prevents oxidative injury by modulating the expression of antioxidant enzyme systems [18]. The oxidative DNA damage in the brain regions of aged rats was also modulated by GSE administration [45]. GSE has been also shown to be protective of nitrosative/oxidative stress [61], and has exhibited superior. From the present result, it is clear that there is improved in liver MDA and PCC contents and increased liver GSH and NO contents in the therapeutics group which received GSE. T. Koga reported that the intake of proanthocyanidins increases the resistance of plasma against oxidative stress and may contribute to physiological functions of plant food through their *in vivo* antioxidant activities 68. Y. Feng demonstrated that treatment with grape seed extracts suppresses lipid peroxidation and reduces hypoxic-ischemic liver injury in the rat 71. Several studies have suggested GSE has a critical role as hepatic protective [38, 55]. Through inhibition of calcium signals and nitric oxide formation [59]. From the previous study as well as the present result, it could be concluded that GSE has a protective and therapeutic role against Tamoxifen toxicity. This is may be the presence of proanthocyanidins and procyanidins that showed antioxidant and free radical scavengers.

A.Cetin reported that grape seed extracted may be promising as a therapeutic option in RTx-induced oxidative stress in the rat liver [1]. I. M. El-Ashmawy concluded that grape seed extracted is a useful herbal remedy, especially for controlling oxidative damages and is considered as a potent protective agent against hepatotoxicity [28]. Several lines of evidence demonstrated that, grapes seed proanthocyanidins exhibited *in vivo* hepatoprotective and anti-fibrogenic effects against liver injury and act as free radical's scavengers and protective liver damage [20].

**CONCLUSION:** The protective and therapeutic action of GSE is observed, this may be due to inhibition of calcium signals and nitric oxide formation, counteracting free radicals, reducing liver toxicity markers. They all contribute to its potential as a modulator of oxidative damage produced by Tamoxifen. Additional studies are needed to demonstrate GSE efficacy in human.

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