Amelioration of tamoxifen-induced liver injury in rats by grape seed extract, black seed extract and curcumin

Hesham A El-Beshbishy, Ahmed M Mohamadin, Ayman A Nagy & Ashraf B Abdel-Naim

Medical Laboratories Technology Dept., Faculty of Applied Medical Sciences, Clinical Biochemistry Dept., Faculty of Medicine Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia

Forensic Medicine and Clinical Toxicology Dept., Faculty of Medicine, Tanta University, Egypt

Pharmacology and Toxicology Dept., Faculty of Pharmacy, King AbdulAziz University, Jeddah, Saudi Arabia.

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Liver injury was induced in female rats using tamoxifen (TAM). Grape seeds (Vitis vinifera) extract (GSE), black seed (Nigella sativa) extract (NSE), curcumin (CUR) or silymarin (SYL) were orally administered to TAM-intoxicated rats. Liver histopathology of TAM-intoxicated rats showed pathologic changes. TAM-intoxication elicited declines in liver antioxidant enzymes levels (glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase), reduced glutathione (GSH) and GSH/GSSG ratio plus the hepatic elevations in lipid peroxides, oxidized glutathione (GSSG), tumor necrosis factor-alpha (TNF-α) and serum liver enzymes; alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase levels. Oral intake of NSE, GSE, CUR or SYL to TAM-intoxicated rats, attenuated histopathological changes and corrected all parameters mentioned above. Improvements were prominent in case of NSE (similarly SYL) > CUR > GSE. Data indicated that NSE, GSE or CUR act as free radicals scavengers and protect TAM-induced liver injury in rats.

Keywords: Curcumin, Free radical seawemger, Grape seed, Liver enzymes, Nigella sativa, Tamoxifen

Tamoxifen citrate (TAM) is non-steroidal antiestrogen drug used in treatment and prevention of hormone-dependent breast cancer. In high dose, it is a known liver carcinogen in rats, due to oxygen radical overproduction and lipid peroxidation via formation of lipid peroxy radicals. Living organisms have developed defense mechanisms for radical detoxification as catalysis of dismutation of superoxide to hydrogen peroxide and oxygen, by superoxide dismutase (SOD) and conversion of H$_2$O$_2$ into water and oxygen by catalase (CAT) or glutathione peroxidase (GPx), which can destroy toxic peroxides. Moreover, glutathione reductase (GR) is considered as antioxidant enzyme reduces glutathione, oxidized by GPx and restores this important component of intracellular redox system.

Intake of oxygen radical scavengers as antioxidants present in plants may be good defense for hepatoprotection, as it contain antioxidant and anticarcinogenic compounds, including phenolics, carotenoids, thiols and tocopherols, which may protect against different diseases. Curcumin (CUR) is yellow phenolic compound present in turmeric; Curcuma longa L. (Family Zingiberaceae) and used as a food preservative. It has been shown to act as antioxidant through modulation of glutathione (GSH) levels and possesses anti-inflammatory properties through interleukin-8 inhibition. Grape (Vitis vinifera) seeds extract (GSE) contains polyphenols including proanthocyanidins and procyanidins, that showed antioxidant and antimicrobial effects, and recently in oral hygiene as it can possess antibacterial activity on oral anaerobes. It has been proved that, free radical scavenging capacity of GSE is 20 times more effective than vitamin E and 50 times more effective than vitamin C. The Nigella sativa, known as “black seed” has been found to possess anti-diabetic activity. It is well known for its hypotensive, hepatoprotective and hypoglycemic effects. It contain highly active quinone compound (thymoquinone) that act as antioxidant used for treatment of diseases including cancer.

The aim of the present work was to study and investigate biochemical effects and antioxidant profile.
of grape seeds extract (GSE), *Nigella sativa* extract (NSE) and curcumin (CUR) on TAM-induced liver injury in rats.

**Materials and Methods**

*Chemicals*—Tamoxifen citrate (TAM), curcumin (CUR), silymarin (SYL) and all other chemicals were obtained from Sigma-Aldrich (USA). Black seeds and grape seeds were purchased from a local authentic herbal store at Al-Madinah Al-Munawwarah. The plant samples were authenticated by the local herbarium located in the faculty of Pharmacy, King AbdulAziz University, Saudi Arabia and samples vouchers were submitted in the herbarium for future use. Diagnostic kits to measure serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma glutamyl transferase (γGT) and alkaline phosphatase (ALP) were obtained from Siemens diagnostics (USA). Diagnostic kit to measure tumour necrosis factor-alpha (TNF-α) in rat liver homogenate were supplied from Biosource Europe, Belgium.

*Animals and treatment*—Adult female Sprague–Dawley rats (120) weighing 120-170 g were obtained from King Fahad Medical Research Centre (KFMRC) Jeddah, KSA. They were kept in cages under standard laboratory conditions (25±2°C; 12 h light/dark) with free access to food and water. Animals were randomly assigned to 10 groups of 12 rats each.

Group 1 (NC)—Normal control rats injected intraperitoneally (ip) with 0.1 ml of normal saline for 21 days.

Group 2 (positive control)—Rats orally administrated SYL for 21 days at a dose of 21 mg/kg/day in normal saline.

Group 3 (TAM-treated rats)—Rats treated with TAM i.p. (45 mg/kg/day in 0.1 ml dimethylsulfoxide and normal saline) for 21 days.

Group 4 (SYL-TAM treated rats)—TAM-intoxicated rats treated with SYL at a dose similar to that for group 2 for 21 days.

Group 5 (GSE-treated rats)—Rats treated with GSE (5g% decoction; 2×250 ml). The liquid administered to rats instead of drinking water (2×250 ml per day) for 21 days.

Group 6 (GSE-TAM treated rats)—TAM-intoxicated rats treated with GSE at a dose similar to that for group 5 for 21 days.

Group 7 (CUR-treated rats)—Rats orally administrated CUR (50 mg/kg/day, dispersed in 0.25% methylcellulose), for 21 days.

Group 8 (CUR-TAM treated rats)—TAM-intoxicated rats treated with CUR at a dose similar to that of group 7 for 21 days.

Group 9 (NSE-treated rats)—Rats treated orally with NSE (800 mg/kg/day in disti. water), freshly prepared for 21 days.

Group 10 (NSE-TAM treated rats)—TAM-intoxicated rats treated with NSE in a dose similar to that for group 9.

At the end of experiment, animals were subjected to light ether anesthesia and blood samples were withdrawn retro orbitally in plain tubes and was centrifuged at 6000 rpm for 10 min within 1 h after collection to separate serum. Anaesthetized animals were killed by cervical dislocation. Abdomen was excised and liver was removed immediately by dissection, washed in ice-cold isotonic saline and blotted between two filter papers. Liver sections were sliced and fixed immediately in formalin for histopathological examination. A 10% (w/v) liver homogenate was prepared in ice-cold 0.1M of potassium phosphate buffer (pH 7.5). Liver homogenate was centrifuged at 5000 rpm for 30 min at 4°C and stored at -80°C for subsequent use.

*Estimation of liver injury through measurement of liver enzymes*—Serum was used to assess hepatic profile enzymes (ALT, AST, γGT, LDH and ALP) using an autoanalyzer (Dimension® Xpand, Siemens Healthcare Diagnostics, Deerfield, IL, USA).

*Estimation of antioxidant profile*—Thiobarbituric acid reactive substance (TBARS) was determined spectrophotometrically as an index of lipid peroxidation using 1,1,3,3-tetraethoxypropane and expressed as nmole TBARS/mg protein. Protein concentration was determined using method of Lowry. Reduced glutathione (GSH) content was estimated spectrophotometrically at 412 nm using 5’-dithio-bis-(2-nitrobenzoic acid) DTNB and concentration was calculated from standard curve as nmole/mg protein. Colorimetric determination of oxidized glutathione (GSSG) was based on the method of Anderson and expressed as nmole/mg protein. Value obtained for GSH was divided by GSSG value to obtain GSH/GSSG ratio. Glutathione peroxidase (GPx) activity was determined spectrophotometrically, and expressed as U/mg protein (1U= nmole of NADPH/min/mg protein). Glutathione reductase (GR) determination was performed through monitoring consumption of NADPH to reduce GSSG. GR activity was expressed as nmole of
NADPH/minute/mg protein (U/mg protein). Catalase (CAT) activity was determined calorimetrically and expressed as μmol H₂O₂ consumed/min/mg protein. Superoxide dismutase (SOD) activity (U/mg protein) was measured spectrophotometrically using xanthine oxidase.

**Determination of tumour necrosis factor-alpha (TNF-α)**—Determination of liver homogenate TNF-α was determined at 450 nm using biotinylated polyclonal antibody to TNF-α and streptavidin conjugated to horseradish peroxidase. TNF-α was calculated by standard curve and expressed as pg/g liver.

**Histopathological examination of liver sections**—Immediately after sacrificing rats, sections from liver were removed and fixed in 10% buffered formalin. Liver slices (0.5 mm thick) were cut. Paraffin embedded sections were cut and stained with hematoxylin/eosin stain (H and E) to examine under light microscope (Olympus BX-50). The liver sections were studied histopathologically in double blind examinations.

**Statistical analysis**—Statistical analysis of obtained data was carried out through analysis of variance (ANOVA) and Student’s t-test. The significance of results was ascertained at P<0.05. Data were expressed as mean ± SEM. The GraphPad InStat and GraphPad Prism software (SanDiego, USA) were used to conduct the statistical analysis.

### Results

**Effect of GSE, NSE and CUR on serum hepatic enzymes of TAM-treated rats**—TAM-intoxication produced significant elevation of serum liver enzymes, AST, ALT, γGT, LDH and ALP compared to normal control rats (Table 1). All these mentioned changes were significantly reduced as compared to TAM-treated rats upon administration of GSE, NSE, CUR or SYL to the TAM-treated rats (Table 1).

**Effect of GSE, NSE and CUR on liver peroxides formation of TAM-treated rats**—TAM administration produced significant increase in liver lipid peroxides formation expressed as thiobarbituric acid reactive substance (TBARS) by 125%, as compared to normal control rats. This elevation was attenuated after administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 39, 60, 42 and 28%, respectively, compared to TAM-intoxicated rats (Fig. 1A).

**Effect of GSE, NSE and CUR on liver non-enzymatic antioxidants of TAM-intoxicated rats**—TAM administration produced significant decrease in liver GSH level by 43%, as compared to normal control rats. This elevation was attenuated after administration of GSE, NSE, CUR or SYL in TAM-intoxicated rats by 39, 60, 42 and 28%, respectively, as compared to TAM-intoxicated rats (Fig. 1B).

### Table 1—Influence of oral administration of GSE, NSE, CUR or SYL on serum hepatic profile enzymes; (ALT, AST, γGT, LDH and ALP) and histology liver injury score of TAM-treated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sAST (U/L)</th>
<th>sALT (U/L)</th>
<th>γGT (U/L)</th>
<th>sLDH (U/L)</th>
<th>sALP (U/L)</th>
<th>Histology liver injury score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal control</td>
<td>110.0±6.47</td>
<td>60.3±0.95</td>
<td>4.0±0.17</td>
<td>380.0±4.5</td>
<td>186.8±4.43</td>
</tr>
<tr>
<td>Group 2</td>
<td>TAM-treated rats</td>
<td>209.0±18.5</td>
<td>88.9±1.6</td>
<td>5.8±0.3</td>
<td>701.0±4.1</td>
<td>252.3±6.13</td>
</tr>
<tr>
<td>Group 3</td>
<td>GSE-treated rats</td>
<td>135.0±9.6</td>
<td>63.3±1.4</td>
<td>4.5±0.19</td>
<td>470.0±2.0</td>
<td>190.1±3.1</td>
</tr>
<tr>
<td>Group 4</td>
<td>GSE-TAM-treated rats</td>
<td>157.4±8.52</td>
<td>76.12±3.4</td>
<td>5.75±0.11</td>
<td>580.0±2.1</td>
<td>220.0±2.1</td>
</tr>
<tr>
<td>Group 5</td>
<td>SYL-treated rats</td>
<td>70.0±3.9</td>
<td>55.0±1.8</td>
<td>4.4±0.10</td>
<td>396.0±1.9</td>
<td>196.3±5.9</td>
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<tr>
<td>Group 6</td>
<td>SYL-TAM-treated rats</td>
<td>128.8±4.5</td>
<td>64.0±1.84</td>
<td>4.6±0.3</td>
<td>480.0±5.0</td>
<td>200.0±2.8</td>
</tr>
<tr>
<td>Group 7</td>
<td>CUR-treated rats</td>
<td>132.8±7.41</td>
<td>68.8±2.1</td>
<td>5.16±0.4</td>
<td>389.0±6.0</td>
<td>170.0±1.9</td>
</tr>
<tr>
<td>Group 8</td>
<td>CUR-TAM-treated rats</td>
<td>180.8±3.9</td>
<td>59.6±2.0</td>
<td>5.25±0.18</td>
<td>430.0±4.0</td>
<td>196.0±1.2</td>
</tr>
<tr>
<td>Group 9</td>
<td>NSE-treated rats</td>
<td>118.7±3.9</td>
<td>55.8±2.0</td>
<td>3.5±0.2</td>
<td>410.0±3.0</td>
<td>182.0±1.3</td>
</tr>
<tr>
<td>Group 10</td>
<td>NSE-TAM-treated rats</td>
<td>134.6±9.3</td>
<td>56.0±2.1</td>
<td>4.75±0.16</td>
<td>510.0±8.4</td>
<td>201.0±1.4</td>
</tr>
</tbody>
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† Significant differences are indicated by P < 0.05, compared with control animals (group 1).

*Significant differences are indicated by P < 0.05, compared with TAM-treated animals (group 2).

Livers were scored for hepatic injury under light microscope with score: 0 = no visible cell damage; 1 = liver damage less than 25% tissue; 3 = extensive damage; and 4 = complete liver damage.
control rats (Fig. 1B). The GSH level was significantly elevated upon administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 28, 36, 33 and 22%, respectively, as compared to TAM-treated rats (Fig. 1B). TAM administration produced significant increase in liver GSSG level by 124%, as compared to normal control rats (Fig. 1C). GSSG level was significantly declined after administration of GSE, NSE, CUR or SYL to the TAM-intoxicated rats by 23, 57, 39 and 27%, respectively, as compared to TAM-treated rats (Fig. 1C). The TAM administration produced significant increase in liver GSH/GSSG ratio level by 31%, as compared to normal control rats (Fig. 1D). This increase was reversed near normalcy up on administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats compared to TAM-treated rats (Fig. 1D).

Effect of GSE, NSE and CUR on liver antioxidant enzymes of TAM-intoxicated rats—TAM administration produced significant decline in liver GPx level by 58%, as compared to normal control rats. GPx level was significantly elevated after the administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 30, 38, 35 and 27% respectively, as compared to TAM-treated rats (Fig. 2A). Similarly, TAM administration produced significant decrease in liver GR level by 54%, as compared to normal control rats. GR level was significantly increased after administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 28, 40, 30 and 21% respectively, as compared to TAM-treated rats (Fig. 2B). TAM administration produced significant decrease in liver CAT level by 83%, as compared to normal control rats. CAT level was significantly elevated after administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 28, 36, 30 and 22%, respectively, as compared to TAM-treated rats (Fig. 2C). TAM administration produced significant decrease in liver SOD level by 53%, as compared to normal control rats. SOD level was significantly raised after administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 31, 37, 31 and 23% respectively, as compared to TAM-treated rats (Fig. 2D).

Effect of GSE, NSE and CUR on liver TNF-α of TAM-intoxicated rats—TAM administration produced significant increase in liver TNF-α level by 415%, as compared to normal control rats. Significantly differences are indicated by *P < 0.05, when compared with control animals. †Significant differences are indicated by †P < 0.05, when compared with TAM-treated animals. NC : Normal control; GSE: Grape seed extract; NSE: Nigella sativa extract; CUR: Curcumin, SYL: silymarin, TAM: Tamoxifen; TBARS: Thiobarbituric acid reactive substance; GSH: Reduced glutathione; GSSG: oxidized glutathione.

Fig. 1—Influence of oral administration of GSE, NSE, CUR or SYL on [A] lipid peroxides formation expressed as TBARS, [B] GSH, [C] GSSG and [D] GSH/GSSG ratio of TAM-intoxicated rat liver homogenate. [Values are expressed as means ± SEM (n= 12). Significant differences are indicated by *P < 0.05, when compared with control animals. †Significant differences are indicated by †P < 0.05, when compared with TAM-treated animals. NC : Normal control; GSE: Grape seed extract; NSE: Nigella sativa extract; CUR: Curcumin, SYL: silymarin, TAM: Tamoxifen; TBARS: Thiobarbituric acid reactive substance; GSH: Reduced glutathione; GSSG: oxidized glutathione].
compared to normal control rats. It was observed that, the TNF-α elevation was significantly reduced after administration of GSE, NSE, CUR or SYL to TAM-intoxicated rats by 39, 56, 43 and 28%, respectively, as compared to TAM-intoxicated rats (Fig. 2E).

Histopathological examination showed preventive effect of GSE, NSE and CUR against liver injury induced by TAM in rats—Stained rat livers were scored for the hepatic injury through examining under the light microscope (50 × magnification), with liver histopathology score (0 – 4). TAM-intoxicated rats elicited the highest score (4) among all groups. CUR elicited lowest score (1) followed by NSE and SYL (score 2) and GSE (score 3) after administration of CUR, NSE, GSE or SYL to TAM-intoxicated rats (Table 1). Liver sections of normal control rats showed no changes (Fig. 3A). TAM-intoxicated liver, showed expanded portal tract, bile duct proliferation, peri-portal apoptosis, mild lymphocytic infiltration and dilated central veins (Fig. 3B) and central veins with regenerated liver nodule, portal tracts showed expansion (Fig. 3C). Liver sections of normal rats administered CUR, NSE, GSE or SYL, showed no changes. Liver of TAM-CUR treated rats, showed mild apoptotic cells, with no pathological changes in either portal system or blood sinusoids (Fig. 3D). Examination of liver sections of TAM-SYL treated rats, showed spotty necrosis (Fig. 3E). Liver sections of rats orally administrated NSE after TAM, showed spotty necrosis and apoptosis (Fig. 3F). Liver section of TAM-GSE, showed regenerated liver nodule, expanded portal tracts, bile duct proliferation, hepatocytes degeneration and mild lymphocytic infiltration (Fig. 3G).

Discussion

This study was carried out in order to investigate role of GSE, NSE and CUR in alleviating liver injury and oxidative stress state produced as a result of TAM-intoxication in female rats. SYL was administered to TAM-intoxicated rats as a reference liver protectant. It has been reported that oxidative stress leads to release of reactive iron ions in liver, generating hydroxyl radicals that react readily with cellular components 30. TAM in toxic doses lead to oxidative liver damage, as it have been elucidated to produce liver injury in rats 31,32. The results obtained from this study demonstrated that TAM administration resulted in significant elevations of sGOT, sGPT, sLDH, sALP and sγGT activities (Table 1) and hepatic TNF-α (Fig. 2E) that were confirmed by abnormal histopathological changes (Fig. 3) and may be attributed to high hepatic levels of TNFα that leads to parenchymal cell apoptosis, inflammation and liver cell necrosis 33. These findings were in accordance with Weber et al. who declared...
that some chemical agents can produce hepatic injury, causing increase serum ALT and AST activities\textsuperscript{34}. All these biochemical changes were significantly improved after NSE, CUR, GSE or SYL administration to TAM-intoxicated rats (Fig. 3; Table 1). These positive changes were prominent in case of NSE (SYL) > CUR > GSE.

Lipid peroxidation is one of the major characteristics that can be included as an oxidative damage marker\textsuperscript{35}. In accordance with the data obtained from this study, TAM administration resulted in significant elevation in TBARS production that may be attributed to the fact that hexose monophosphate shunt in rat liver is strongly inhibited by high dose of TAM, so that NADPH levels inside cells is decreased\textsuperscript{31,32}. The level of TBARS were significantly decreased compared to TAM-intoxicated group (Fig. 1A) upon administration of GSE, NSE, CUR or SYL. To further substantiate the antioxidant activity of CUR, NSE and GSE, the activities of the intracellular antioxidant enzymes were assessed. In our study, hepatic antioxidant enzymes, GR, GPx, CAT and SOD were significantly reduced in TAM-treated rats. Oxidative stress noticed after TAM intoxication was associated with decreased hepatic GSH, GR content and increased peroxidation concomitant with high level of GSSG\textsuperscript{36}. SOD, CAT and GPx were decreased after initiation of lipid peroxidation process\textsuperscript{31,32}, which were in accordance with results achieved from this study. It was reported that, CAT showed no significant change after TAM intoxication\textsuperscript{37}. Decreased GPx level of TAM-intoxicated rats, leads to an increase of the oxidative stress of the cells\textsuperscript{30}. It was reported that, CUR and GSE that enhance their effectiveness as powerful antioxidants involved the enhanced synthesis of the endogenous antioxidant enzymes SOD, CAT and GPx\textsuperscript{38,39}. Decreased activities of hepatic GPx, CAT and SOD of TAM-intoxicated rats may be due to oxidative stress induced inactivation and/or exhaustion\textsuperscript{40}, as the decreased hepatic GPx activity may leads to H$_2$O$_2$ accumulation in the liver which in turns inactivates SOD\textsuperscript{41}. The impaired regeneration of protective and antioxidants such as GSH and GR also contribute to oxidative stress\textsuperscript{42}. Furthermore, improvement of phase II detoxifying and antioxidant enzymes and elevation of antioxidant substance content is one of the mechanisms to ameliorate antioxidant status through sulfation, glucuronidation and glutathiolation to neutralize electrophilic metabolites\textsuperscript{43}.

The decreased hepatic GSH in TAM-intoxicated rats was attributed to hexose monophosphate shunt impairment due to TAM-intoxication and thereby NADPH availability is reduced and ability to recycle GSSG to GSH is decreased\textsuperscript{44}. Our results showed that,
activities of hepatic GPx and GR were significantly reduced upon TAM-intoxication when compared to control group (Fig. 2). It has been shown that CUR, NSE and GSE can protect against GSH depletion by sequestering reactive oxygen species and increases GSH synthesis\(^{45}\), as GSH regulates expression of antioxidant genes. The level of hepatic antioxidant enzymes levels were significantly improved upon treatment of TAM-intoxicated rats with GSE, NSE or CUR (Fig. 1, 2). These changes were prominent in case of NSE (and similarly SYL) > CUR > GSE. It has been shown that CUR, NSE and GSE are effective antioxidants and have contributory roles in protecting cells from DNA damage\(^{46,47,48}\). In agreement with the results obtained in this study, it was reported that, administration of green tea (contain polyphenols) to TAM-intoxicated rats, resulted in normalization of lipid peroxidation\(^{31}\) as well as GSH and GPx activity in liver that was attributed to the induction of SOD, which present at low level only but highly inducible under oxidative stress\(^{49}\). It is thought that effectiveness of polyphenols as free radical scavengers attributed to their structure. Phenolic and methoxy groups on CUR benzene rings and 1,3-diketone system are important for oxygen free radical scavenging\(^{50}\). GSE has been shown to have conjugated structures between the B-ring catechol groups and 3-OH free groups of polymeric skeleton allowing to be effective free radical scavengers and metal chelators\(^{51}\). As GSE scavenges free radicals, the resulting aroxyl radical formed has been shown to be more stable than those generated from other polyphenolics to prevent DNA damage\(^{52}\).

TNF-\(\alpha\) is a proinflammatory cytokine that is associated with liver injury in many experimental models\(^{53}\), and human diseases\(^{54,55,56}\). In the current study, treatment of rats with TAM resulted in a 4-fold increase in TNF-\(\alpha\) level. In accordance with our results, it was reported that TAM administration resulted to rats in a significant increase in TNF-\(\alpha\) level in rats\(^{52}\). Additional support comes from the reports indicating that pathology of TAM-induced liver injury includes inflammation resembling that of alcoholic hepatitis\(^{57}\). However, pretreatment with CUR, NSE or GSE significantly inhibited the rise in the TNF-\(\alpha\) level. This is consistent with our histopathological findings that indicated decreased lymphocytic infiltration in liver tissues of CUR, NSE or GSE-treated animals. Further, this is also consistent with the known anti-inflammatory activity of CUR, NSE or GSE. Also, our data indicated that, TAM induced histopathological changes in liver tissues. These included bile duct proliferation, lymphocytic infiltration, edema and hepatocytes degeneration, CUR, NSE or GSE clearly ameliorated the histopathological changes. CUR elicited low histology liver injury score followed by NSE and GSE. These findings support our results showing the hepatoprotective antioxidant activities elicited by CUR, NSE and GSE.

In conclusion, data achieved from this study revealed that, the pre-treatment with NSE, CUR or GSE protects against TAM-induced hepatic injury by preserving cellular integrity, preventing oxidative stress and lipid peroxidation, enhancing antioxidant enzymes activities and inhibition of the hepatic inflammation.

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