Thioredoxin1 Gene Modulates Bcl2/p53/NF-KB Signaling Pathways in Strawberry Extract/Paracetamol-treated Rat Model of Acute Liver Injury

Aysam Fayed¹, Hala O. Ramadan¹, Soha A. Hassan², Mohammed A. Hussein³* and Tamer Roshdy¹

¹Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Menoufia, Egypt.
²Basic Science Department, Faculty of Dentistry, October 6 University, Sixth of October City, Egypt.
³Biotechnology Department, Faculty of Applied Medical Health Sciences Technology, October 6 University, Sixth of October City, Egypt.
*Corresponding Author E-mail: prof.husseinma@o6u.edu.eg

https://dx.doi.org/10.13005/bpj/2438

(Received: 29 November 2021; accepted: 11 March 2022)

When used in excess, the analgesic paracetamol can cause hepatic centrilobular necrosis, which can be fatal. The goal of this study was to see if strawberry extract could protect rats' livers from paracetamol-induced hepatotoxicity. Strawberry (75 and 150 mg/kg bw) and vit C (1 g/kg bw) were given orally, daily for 15 days demonstrated a significant reduction in the effects of caused changes in plasma cholesterol, triacylglycerol, phospholipids and vit C, TBARS, GSH, TNF-α, IL-4 and NO, AST, ALT, ALP, LDH, SOD, GPx and GSH levels. Furthermore, strawberry extract significantly inhibits hepatocyte B-cell lymphoma 2 (Bcl2) but significantly induces p53, NF-KB and Trx1 gene expression compared to paracetamol-treated rats. Histological examination showed that significant normalization has been observed in strawberry extract treated rats. Conclusions Strawberry extract shows considerable hepatoprotective benefits in the case of paracetamol-induced liver damage, confirming it's essential use as a treatment for liver damage.

Keywords: Hepatoprotective; Inflammatory Mediators; Oxidative Stress Biomarkers; Paracetamol; Strawberry.

Liver, as the core of metabolic processes, has role to perform in the metabolism of a wide range of xenobiotics and is more susceptible to their toxicity¹. Acetaminophen overdoses, which are both analgesics and antiparasitic, are the most common poisonings caused by pharmaceutical products in the U.S. now². Despite being regarded safe at moderate dosages, Overdosing on acetaminophen leads to centrilobular liver necrosis, which can be deadly³. While early metabolism events of toxicity have been well described, Hepatocyte death's precise mechanisms are unknown. Necrosis is the term for cell death, and apoptosis has been ruled out⁴.

Acetaminophen is normally converted to an active intermediate, NAPQI, by cytochrome P450 enzymes. It was quickly detoxified by glutathione conjugation⁵. Excessive NAPQI binds to mitochondrial proteins in hepatocellular, destroying mitochondria and causing a flood of reactive oxygen species, peroxidation, eventually liver cell death. Natural
antioxidants obtained from various alternative medicine systems have been shown in numerous studies to have a wide spectrum of biological actions\(^6-9\). In animal models, a variety of antioxidant-rich alternatives have been utilised to reduce paracetamol-induced oxidative stress\(^8\). Several plant extracts were shown to be beneficial in reducing organ toxicity\(^6-10\). Strawberry is one of these plants. Strawberry is a crop grown all over the world, with different cultivars suited to different climates\(^11\). It is the best source of minerals like mn, k, mg, cu, fe, and p\(^12\), ascorbic acid\(^13\), thiamine, riboflavin, niacin, vit B6, vit K, vit A, and vit E\(^14\), folate\(^15\), catechins, hydroxycinnamic acids, ellagitannins and ellagic acid have also been associated with the beneficial effect of strawberries on human health\(^16\). Ascorbic acid, ellagitannins, and anthocyanins are the most important contributors to strawberries’ antioxidant capacity\(^17\). Hepatoprotective\(^18\), hypolipidemic\(^19\), hypoglycemic, and antioxidant activities of extract berry fruits have been studied in vivo\(^20\). Strawberry extract, on the other hand, has not been shown to have hepatoprotective or gastroprotective properties. We want to test therapeutic potential of strawberry extract on paracetamol-induced liver damage in rat model as a follow-up to our studies on biological value of neutral products\(^19-24\).

**Materials and Methods**

**Chemicals**
- El-Nile Pharmaceutical Company gave us paracetamol as a present (Cairo, Egypt). When used in vivo experiments, paracetamol was suspended in 0.5 % tween 80 and administered orally at a dose of 1 g/kg PB\(^25\).
- Prolabo and Farance collaborated on Tween 80.
- Virgin Extracts (TM) in China provided the strawberry extract. Cranberry extract (75 and 150 mg/kg bw) was given to rats using an oral gastric gavage tube twice a day for two weeks.

**Animals**
Adult albino rats measuring roughly 20010 gms. They had been accustomed to the confines of an animal shelter. The animals were fed a regular feed and given free access to water. All through the trial, the rats were housed in the same habitat and were observed on a daily basis.

**Experimental setup**
The purpose of this study was to see if ethanolic and aqueous extracts of cranberry extract could prevent paracetamol hepatotoxicity in vivo when given periodically for two weeks.

The following treatments were given on a daily basis for 14 days. To intragastric intubation of rats, a 3 percent suspended solution was created.

**Group I**: Typical (Orally given a similar volume of tween 80, 1 percent in saline)

**Group II**: Control (Orally administered a similar volume of tween 80, 1 percent in saline)

**Group III**: Received a single daily dose of strawberry extract suspended in tween 80\(^19\).

**Group IV**: Strawberry extract suspended in saline (150 mg/kg bw) was given orally in a single daily dose\(^19\).

**Group V**: vit C (1 g/kg bw) orally as a single daily dose suspended in tween 80\(^19\).

Through day 13, the day prior to final treatment. Animals belonging to categories II, III, IV, and V received on day 14, one hour after the last dosage of pharmaceutical therapy, paracetamol\(^26\).

**Treatment of blood samples**
Samples of blood were collected in heparin-containing tubes from each animal’s retroorbital vein. Blood was heparinized and centrifuged at 1000 xg for 20 minutes cholesterol\(^27\), triglycerides\(^28\), (HDL)\(^29\), phospholipids\(^30\), ALT\(^31\), AST\(^31\), ALP\(^32\), LDH\(^33\) and vit C\(^34\) levels were measured in separated plasma.

**Preparation of liver samples**
These animals were killed using cervical dislocation, livers were extracted swiftly. To make a homogenate with a 25 percent W/V ratio, Using glass homogenizer, a fraction of each liver was homogenised with saline and weighed. The homogenate was produced in three aliquots. The first was used 12 percent trichloroacetic acid, chilled on ice, and the resulting supernatant was employed for GSH measurement after centrifuged at 1000 xg\(^35\). The second was rotated at 1000 xg and resulting supernatant was used to estimate the levels of TBARS\(^36\), NO\(^37\), TNF-α\(^38\) and IL-4\(^39\). The third was utilised create cytosolic fraction of liver using a cooling ultracentrifuge at 10500 xg for 15 minutes at 4 OC, and the clear supernatant was used to evaluate SOD\(^40\) and GPx\(^41\).
Real-time PCR

The manufacturer’s instructions were followed to obtain total hepatic RNA using the TRizol technique (Life Technologies Corp., Grand Island, NY). 1 µg RNA was combined with 0.5 mmol/l each deoxyribonucleoside triphosphate, 10 mmol/l dithiothreitol, 25 pg oligo (dT) primer (dNTP), and 200 units of superscript II Rnase H reverse Transcriptase in reaction buffer. The reactions have been incubated for one cycle for 2 min at 42° C and again 50 min at 42° C, which they were heated for 15 min at 70°C and then chilled to 4° C.

(Table 2): Bcl2, P53, nuclear factor kappa, and Trx1. The PCR reaction mixtures were incubated for 3 minutes at 94° C, then for the appropriate number of cycles at 94° C for 45 seconds, then at their respective annealing temperatures for 30 seconds, and for 30 seconds at 72° C. After that, a 10 minute extension step at 72° C was performed.

Histological assessment

Liver cut to little fragments then stored in formaldehyde solution containing 10% buffered formaldehyde. Under the microscope, the slices were evaluated for histological alterations.

Statistical analysis

To get mean, standard deviation, and error, the data analysed using statistical package for social science. To establish statistical significance of differences between groups, data were analysed using one-way analysis of variance. Duncan’s test was examine intergrouping homogeneity by doing multiple comparisons among the groups.

RESULTS

Table 1 When contrasted to normal group, oral administration of paracetamol at 1g/kg bw. caused increase in plasma cholesterol and triglycerides, In addition drop in HDL-C and phospholipids (p < 0.01). When contrast to the group receiving paracetamol, supplementation with strawberry extract (75 and 150 mg/kg bw) and Vit C (1 g/kg bw) as a result of substantial drop in plasma cholesterol and triglycerides, In addition rise in HDL-C and phospholipids (p < 0.05). Strawberry has dose-dependent impact (p < 0.05).

Table 2 contrasted to normal group, oral administration of paracetamol at 1g/kg bw as a result of substantial increase in plasma ALT, AST, ALP, and LDH, In addition drop in vit C levels (p < 0.01). When contrast to group receiving paracetamol, supplementation with strawberry extract (75 and 150 mg/kg bw) and vit C (1 g/kg bw) as a result of substantial decrease in plasma ALT, AST, ALP, and LDH, In addition increase in vit C levels (p < 0.05).

Table 3 contrasted to control group (p < 0.01), orally administered paracetamol at dose of 1 gramme per kilogramme of body weight was shown as a result of substantial rise in lipid peroxides in the liver (TBARS). Strawberry extract (75 and 150 mg/kg bw) and vit C (1 g/kg bw) supplementation as a result of substantial reduction in liver TBARS when compared to paracetamol group (p <0.05). Furthermore, as compared with control group, orally administered paracetamol as a result of substantial decrease in reduced GSH, SOD, and GPx in liver (p < 0.01). When contrast to group

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl2</td>
<td>F: 5'-TGTGGATGACTGACTACCTGAACC3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CAGCCAGGAGAAATCAAACAGAGG3'</td>
</tr>
<tr>
<td>p53</td>
<td>F: 5'-CTACTAAGGTCGTAGACGCTGCC3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-TCAGCATACAGGTTTCCCTTCCACC3-</td>
</tr>
<tr>
<td>NF-KB</td>
<td>F: 5'GCAAACCTGGGAAATCTCTATGTGACTAG3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'ATAGGCAAGGTCGAAGTGCACACAGTCC3'</td>
</tr>
<tr>
<td>Trx1</td>
<td>F: 5'-CCGCAACAGCCAAAATGTTTG3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-AGCATGATTAGCACAATTCCCTGG3-</td>
</tr>
<tr>
<td>β-Actin (internal control for qRT-PCR)</td>
<td>F: 5'-GGCTGTATTTCCCTTCCATCG3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CCAGTTGGTAAACATGCCCAGT3'.</td>
</tr>
</tbody>
</table>
Table 2. Shows effects of triglycerides, HDL cholesterol, and phospholipids in rats.

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>Plasma triglycerides (mg/dl)</th>
<th>Plasma HDL-C (mg/dl)</th>
<th>Plasma phospholipids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal 1% tween 80</td>
<td>172.67± 9.22a</td>
<td>105.79± 9.97a</td>
<td>37.14± 5.16 a</td>
<td>61.12± 6.12 c</td>
</tr>
<tr>
<td>(II)</td>
<td>Control Paracetamol (1 g/kg.b.w)</td>
<td>202.48± 7.48c</td>
<td>177.80± 10.98d</td>
<td>28.13± 4.56c</td>
<td>36.70± 3.45a</td>
</tr>
<tr>
<td>(III)</td>
<td>Paracetamol + Strawberry extract (75 mg/kg.b.w)</td>
<td>180.46± 7.79b</td>
<td>135.13± 10.90c</td>
<td>32.79± 4.24b</td>
<td>47.17± 4.36b</td>
</tr>
<tr>
<td>(IV)</td>
<td>Paracetamol + Strawberry extract (150 mg/kg.b.w)</td>
<td>170.56± 9.98a</td>
<td>106.40±9.98a</td>
<td>38.26± 2.62 a</td>
<td>59.07± 3.70 c</td>
</tr>
<tr>
<td>(V)</td>
<td>Paracetamol + Vitamin C (1 g/kg.b.w)</td>
<td>169.29± 8.93a</td>
<td>118.59± 7.20 b</td>
<td>34.38± 3.21a</td>
<td>62.95± 5.35c</td>
</tr>
</tbody>
</table>

To 18h fasted animals, a single dosage of 1g/kg.b.w. of paracetamol was orally. Except for the regular group, it was provided to everyone else. For two weeks, strawberry extract and vitamin C on plasma cholesterol, were given orally, with the last dose of each given one hour before paracetamol. The numbers of the mean (n=6). numbers displayed are the mean and standard deviation of the number of observations in all treatment. At P = 0.05, data following the same letter are not substantially different.

Table 3. Shows the effects of strawberry extract and vitamin C in rats given on plasma ALT, AST, ALP, LDH, and vit C

<table>
<thead>
<tr>
<th>No</th>
<th>Groups</th>
<th>Plasma ALT (U/L)</th>
<th>Plasma AST (U/L)</th>
<th>Plasma ALP (U/L)</th>
<th>Plasma LDH (U/L)</th>
<th>Plasma Vit C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal 1% tween 80</td>
<td>27.10± 3.81a</td>
<td>15.98± 2.54a</td>
<td>154.15± 950 a</td>
<td>309.45± 10.32 a</td>
<td>83.49± 7.02 d</td>
</tr>
<tr>
<td>(II)</td>
<td>Control Paracetamol (1 g/kg.b.w)</td>
<td>60.48± 5.05d</td>
<td>433.8± 3.87 d</td>
<td>2893±14.98 e</td>
<td>5373± 14.3 1e</td>
<td>30.79± 4.45 a</td>
</tr>
<tr>
<td>(III)</td>
<td>Paracetamol + Strawberry extract (75 mg/kg.b.w)</td>
<td>43.88± 452 c</td>
<td>32.24± 6.08 c</td>
<td>207.49±10.58d</td>
<td>392.48± 12.39 d</td>
<td>67.42± 4.48 b</td>
</tr>
<tr>
<td>(IV)</td>
<td>Paracetamol + Strawberry extract (150 mg/kg.b.w)</td>
<td>31.73±3.38a</td>
<td>21.98±4.65b</td>
<td>171.69±12.18 b</td>
<td>340.96±17.73 b</td>
<td>7526±4.38 c</td>
</tr>
<tr>
<td>(V)</td>
<td>Paracetamol + Vitamin C (1 g/kg.b.w)</td>
<td>35.19±3.08b</td>
<td>3154±3.80c</td>
<td>189.30±7.15c</td>
<td>364.25±15.94c</td>
<td>80.41±4.44d</td>
</tr>
</tbody>
</table>

To 18h fasted animals, a single dosage of 1g/kg.b.w. of paracetamol was given orally. Except for the regular group, it was provided to everyone else. For two weeks, strawberry extract and vitamin C were given orally, with the last dose of each given one hour before paracetamol. The numbers of the mean (n=6). numbers displayed are the mean and standard deviation of the number of observations in all treatment. At P = 0.05, data following the same letter are not substantially different.
Table 4. Shows the effects of strawberry extract and Vitamin C on TBARS, GPx, SOD, and GSH in rats.

<table>
<thead>
<tr>
<th>No. Groups</th>
<th>TBARS (mmol/mg wet tissue)</th>
<th>GSH (mg/g tissue)</th>
<th>SOD (U/gm tissue)</th>
<th>GPx (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Normal 1% tween 80</td>
<td>1.44±0.36a</td>
<td>45.17±5.27d</td>
<td>26.60±3.88e</td>
<td>153.72±5.40d</td>
</tr>
<tr>
<td>(II) Paracetamol (1 g/kg.b.w)</td>
<td>5.39±0.51e</td>
<td>23.76±5.30a</td>
<td>12.0±2.11a</td>
<td>55.19±3.09a</td>
</tr>
<tr>
<td>(III) Paracetamol + Strawberry extract (75 mg/kg.b.w)</td>
<td>3.79±0.52m</td>
<td>3.16±0.22b</td>
<td>18.87±2.30b</td>
<td>132.14±7.01b</td>
</tr>
<tr>
<td>(IV) Paracetamol + Strawberry extract (150 mg/kg.b.w)</td>
<td>2.66±0.33b</td>
<td>40.99±4.38c</td>
<td>25.39±3.41c</td>
<td>148.23±9.12c</td>
</tr>
<tr>
<td>(V) Paracetamol + Vitamin C (1 g/kg.b.w)</td>
<td>3.21±0.38c</td>
<td>43.63±4.42c</td>
<td>17.41±1.89b</td>
<td>136.28±8.16b</td>
</tr>
</tbody>
</table>

To 18 h fasted animals, a single dosage of 1 g/kg bw. of paracetamol was given orally. Except for the regular group, it was provided to everyone else. For two weeks, a single dosage of strawberry extract (75 and 150 mg/kg bw) and VIT C (1 mg/kg bw) were given orally, with the last dose of each given one hour before paracetamol. Numbers of the mean (±S.D), mean and standard deviation of number of observations in all treatment are presented. At $P = 0.05$, data not receiving same letters are not substantially different.

that received paracetamol, supplement with strawberry and vit C substantial increase in GSH, SOD, and Gpx ($P < 0.05$). Strawberry 150 mg/kg of strawberries has a stronger effect than vit C ($P < 0.05$).

Table 4 contrasted to control group, orally administered paracetamol at 1 g/kg bw. a result of substantial rise TNF-α, NO, and IL-4 ($P < 0.01$). When compared to group that received paracetamol, orally administered strawberry (75 and 150 mg/kg bw) and Vit C (1 mg/kg bw) a result of substantial decrease TNF-α, NO, and IL-4 ($P < 0.05$). Strawberry 150 mg/kg had a stronger effect than vit C ($P < 0.05$).

Figures 1-4 show significant decrease in expression of hepatocyte Bcl2 in addition significant increase in expression of p53, NF-KB and Trx1 genes. the management of rats with strawberry extract (75 and 150 mg/kg bw) and vit C (1 g/kg bw) showed significant significant increase in Bcl2 in addition significant decrease in liver p53, NF-KB, and Trx1, compared to paracetamol-treated rats ($P < 0.01$).

The normal group’s liver slices were examined histopathologically (I) showed within normal arrangement and appearance of hepatocytes without fibrosis or inflammation x 200 H&E (Figure 4a).

However, in liver of paracetamol-treated control group (II), showed a greatly altered hepatic parenchyma due to diffuse hydropic degeneration and vacuolar degeneration in hepatocytes (yellow arrows), narrowed or occluded sinusoids with congested central veins (CV) and abnormal arrangement of the blood sinusoid (red arrows) (high magnification X: 400 bar 50 (figure 4b). Furthermore, the hepatocytes showed mildly disrupted hepatic parenchyma due to lesser degrees of hepatocyte degeneration (yellow arrows), some blood cells (#) between hepatocytes in some lobules and mild congested sinusoids (red arrows) in paracetamol-treated rats administrated with strawberry extract (75 mg/kg bw) as compared to the paracetamol-treated group (figure 4c) group (III) (high magnification X: 400 bar 50). Also, the histological examination of hepatocytes of rats treated with paracetamol + strawberries (150 mg/kg bw) groups (IV) showed mild lobular inflammation (yellow arrows) and lesser degrees degeneration of hepatocytes, also nearly normal.
blood sinusoids(S) and normal portal area, (high magnification X: 400 bar 50) (Figure 4d). Also, Microscopic picture of H&E stained liver sections (I, J) showing improvement in histological morphology histological structure of the liver lobes, natural hepatocyte arrangements (*) with intact blood sinusoids (S) and normal portal area in rats administered treated with paracetamol administered vitamin C (1g / kg) (Figure 4e) (high magnification X: 400 bar 50).

**DISCUSSION**

Paracetamol (4'-Hydroxyacetanilide) is antipyretic and analgesic medication that is used orally. Sulfonation, glucuronidation, and oxidation are the three primary mechanisms by which it is metabolised by liver. The first two processes are more essential in terms of quantity than the third, but the oxidative pathway is the source of toxicity. Hepatic microsomes catalyse the oxidation of paracetamol, which is predominantly mediated by cytochrome P-450. The procedure yields NAPQI, a highly reactive arylating chemical. The P-450A2 microsome was found to be the major catalysts of paracetamol activation in the human liver. When many NAPQI is created can be conjugated to GSH, unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins.

**Table 5. Shows effects of strawberry extract and vitamin C on rat liver nitric oxide (iNOs), TNF-α, and IL-4 levels**

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Hepatic iNOs (pg/mg tissue)</th>
<th>Hepatic TNF-α (pg/mg tissue)</th>
<th>Hepatic IL-4 (U/g.m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal 1% tween 80</td>
<td>3.05±0.42 a</td>
<td>19.32±2.88 a</td>
<td>3.197±4.90 a</td>
</tr>
<tr>
<td>(II)</td>
<td>Control Paracetamol (1 g/kg.b.w)</td>
<td>8.18±0.45 b</td>
<td>54.59±5.84 c</td>
<td>78.06±5.70 c</td>
</tr>
<tr>
<td>(III)</td>
<td>Paracetamol + Strawberry extract (75 mg/kg.b.w)</td>
<td>5.38±0.87 a</td>
<td>34.07±3.28 b</td>
<td>44.25±5.48 b</td>
</tr>
<tr>
<td>(IV)</td>
<td>Paracetamol + Strawberry extract (150 mg/kg.b.w)</td>
<td>3.12±0.46 a</td>
<td>25.36±3.50 a</td>
<td>33.63±4.0 la</td>
</tr>
<tr>
<td>(V)</td>
<td>Paracetamol + Vitamin C (1 g/kg.b.w)</td>
<td>4.20±0.85 a</td>
<td>35.07±2.99 a</td>
<td>49.45±4.77 b</td>
</tr>
</tbody>
</table>

To 18h fasted animals, a single dosage of 1g/kg.b.w. of paracetamol was given orally. Except for the regular group, it was provided to everyone else. For two weeks, strawberry extract and vitamin C were given orally, with the last dose of each given one hour before paracetamol. The numbers of the mean (n=6), mean and standard deviation of the number of observations in all treatment are presented. At P 0.05, data following the same letter are not substantially different.

**Fig. 1.** In paracetamol-treated rats, effect of strawberry extract and vit C on levels of liver (Trx1) gene expression
Plant-based medicine Medications may contain phytoconstituents that occur naturally, which can decrease production of reactive oxygen species in variety of ways. Pharmacological effects of phytoconstituents are diverse\textsuperscript{51, 52}.

The treatment of paracetamol to rats resulted increase in the levels of cholesterol, triglycerides, AST, ALT, ALP, LDH, iNos, TNF-\(\alpha\), IL-4 and TBARs levels, however, decrease in vit C, HDL-C and phospholipid levels as well as GSH, SOD and GPx contrasted to normal rats. Orally administration extract and vit C to paracetamol treated rats showed normalization of plasma levels of cholesterol, triglycerides, AST, ALT, ALP, LDH, iNos, TNF-\(\alpha\), IL-4, TBARs vitamin C, HDL-C, GSH, SOD and GPx levels when contrasted to paracetamol treated rats\textsuperscript{53}. reported that paracetamol administration caused AST and ALT activities were elevated.

Because strawberry possesses scavenging free radical damage and antioxidants\textsuperscript{41}, goal investigation is to see if cranberry polyphenols may reduce diclofenac-induced liver damage in rats. Strawberry extract considerably reduces the peroxidative damage caused by diclofenac sodium in liver, as demonstrated by decreased levels of reactive chemicals thiobarbituric acid and lipid hydroperoxides could be related to polyphenols’

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{In paracetamol-treated rats, effect of strawberry extract and vit C on levels of liver NF-kB gene expression}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{In paracetamol-treated rats, effects of strawberry extract and vit C on levels of liver P53 gene expression}
\end{figure}
antioxidant properties\textsuperscript{42}. Antioxidant is a chemical that can slow or stop other molecules from oxidising. Oxidation reaction in which electrons are transferred from material oxidizer. The lipid peroxidation mechanism causes cellular damage and functional problems in hepatocytes in response to paracetamol\textsuperscript{7}. Because the liver is involved in lipid homeostasis, increased iron may affect serum lipid concentrations, thereby reducing or increasing risk of atherosclerosis. Strawberry was found to be non-toxic in normal rats in preliminary investigations undertaken by this work. Hepatic necrosis after a large dose of paracetamol has been widely established\textsuperscript{53}. The current investigation found a significant increase in ALT, AST, ALP, and LDH after paracetamol administration (1g/kg bw) because of Ca\textsuperscript{2+} buildup, which activated phosphofructokinase and anaerobic glycolysis, culminating in lactate production\textsuperscript{54}.

Fig. 4. In paracetamol-treated rats, effect of strawberry extract and vit C on levels of liver Bcl2 gene expression

Fig. 5. Histological examination of mice’s lung tissues stained with eosin and hematoxylin (H&E; 400 X) when contrasted to the control group
The last stage of the paracetamol cell death process has been defined as the loss of Ca2+ homeostasis induced by oxidative damage and increase in intracellular Ca2+. Strawberry treatment regulates plasma LDH activity. Both light and TEM examinations demonstrated that indomethacin and strawberry extract cause inflammatory in hepatic and gastric mucosal cells. This finding backs up the fact that rats given strawberry extract with paracetamol had lower levels of TNF-α, NO, IL-4, and thiobarbatic acid (TBARS) in their livers. Our results indicated that the hepatoprotective effect of strawberry extract could be due to the presence of ascorbic acid, thiamine, riboflavin, niacin, vit B6, vit K, vit A and vitamin E, folate, flavonols, catechins, hydroxycinnamic acids, ellagitannins and ellagic acid.

The strawberry extract-treated groups had considerably lower liver TBARS levels than the paracetamol-treated groups in this study. Strawberry extract may have antioxidant properties and protect tissues from lipid peroxidation, according to this finding. Protective effect of strawberry extract treatment strongly suggested that extracts are capable of stopping the release of marker enzymes to the bloodstream, conditioning Hepatocytes help to speed up the regeneration of parenchymal cells, and preserving integrity plasma membranes and thus restoring enzyme activities.

SOD and GPx activity in the blood and liver were shown to be lower in rats given paracetamol. SOD and GPx activity were found to be lower in paracetamol-treated mice by several authors. The findings suggest that strawberry extract can either boost SOD and GPx production or decrease inflammation, resulting with less SOD and GPx breakdown, or both. Hepatotoxicity from paracetamol manifests as centrilobular necrosis. Biochemical findings are backed up by histopathological research. Fatty alterations, necrosis, and vacuoles. To paracetamol-treated rats, orally administration strawberry (75 and 150 mg/kg bw) and vit C. In paracetamol-treated rats, researchers found vacuolated hepatocytes, necrosis, and congested sinusoids in the liver. The injection of - and -amyrin to the liver of paracetamol-treated rats exhibited normal histoarchitecture, according to Oliveira et al. Presence of flavonoids was discovered during a phytochemical screening of strawberry extract. Flavonoids (or bioflavonoids) are natural compounds impact the behaviour numerous cell systems through enzyme activity (SOD and GPx) and have antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic, anticancer, and antioxidant properties.

Oxidative stress plays role in hepatotoxicity paracetamol. Induction of oxidative stress may suppress Bcl2 and trigger p53 activation, as well as induction of the expression of the NF-KB and Trx1 genes. The Trx1 system is a sensor of energy and glucose metabolism and contributes to cellular redox equilibrium. Our study suggested that the overexpression of Trx1 in rats treated with paracetamol to modulate the redox state and liver cells and provide Cys for the synthesis of protein and coenzyme A, this Cys source is used for the de novo synthesis of GSH, thus putting the reduced sulfur derived from Met in a molecule that can support reductions in cytosolic disulfides. However, the results obtained explain that the administration of strawberry extract containing...
polyphenols and flavonoids can negatively regulate Trx1 gene expression, its free radical scavenging activity, and the induction of GSH biosynthesis. p53 plays a role in a variety of diseases. Evidence does, however, support a pro-survival function in specific circumstances. Paracetamol poisoning has been demonstrated to activate p53.

Activation of NF-κB and p53 serves as a signal to trigger cytotoxicity. However, increasing body of evidence supports function of NF-κB and p53. Oxidative stress can induce activation of p53. In fact, p53 is activated in response to paracetamol exposition in animal models. Present, the administration of strawberry containing polyphenols and flavonoids can down-regulate NF-κB and p53 by reducing oxidative stress. Strawberry extract suppresses regulation of hepatic Bcl-2 overexpression in animal model of paracetamol-induced acute liver damage, according to this study. Our findings, which coincide with Boulares et al., reveal that paracetamol overdose inhibits anti-apoptotic Bcl-2. Furthermore, our findings are consistent with at least two studies found that hypoxia-ischemia inhibited Bcl-2 levels in rat model of neonatal brain injury and mouse model of paracetamol-induced liver injury, respectively, and that resveratrol treatment substantially increased Bcl-2 levels.

Strawberry extract, according to histopathological studies, can protect the liver from paracetamol-induced liver damage. A diminished inflammatory response is proof of this. Strawberry’s protective effect against paracetamol-induced liver injury has not before been described, and could be the first study of its kind.

Finally, the findings of this investigation showed that strawberry extract has a significant hepatoprotective effect in rats when exposed to paracetamol. This could be because of its antioxidative properties, which include the capacity to reactive oxygen species scavenge and prevent peroxidation of lipids.

Consent for Publication
The authors gave consent for their data to be used in the article.

Competing interests
The authors declare no conflict of interest, financial or otherwise.

Authors’ contributions
Experimental design, antioxidant and hepatoprotective activities of strawberry water extract were carried out by Hala O Ramadan, Aysam Fayed and Tamer Roshdy. Histopathological examination was carried out by Soha A. Hassane. Wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study, managed the literature searches was carried out in collaboration between Hala O Ramadan, Aysam Fayed, Mohammed A. Hussein and Tamer Roshdy. All authors read and approved the final manuscript.

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