Grapefruit juice (GFJ) is a rich source of various nutritional components, including vitamin C and polyphenols, and has some claimed therapeutic properties (1). However, it has been shown to interact with several commonly prescribed drugs (2). It has been shown, for example, to interact with antibiotics such as erythromycin (3), antilipemic agents such as simvastatin and atorvastatin (4), antihypertensive agents such as nifedipine (5), and immunosuppressant agents such as cyclosporine and tacrolimus (6). These interactions can alter the systemic concentration of these drugs. Some of these interactions might cause significant clinical complications such as torsade de points with amiodarone (7), rhabdomyolysis with atorvastatin (4), severe hypotension with calcium channel blockers and nephrotoxicity with tacrolimus (6).

The inhibition of intestinal cytochrome P450 enzymes is the reported principal mechanism by which GFJ exerts its interaction with these drugs (8-9). GFJ has also been reported to inhibit hepatic drug-metabolizing enzymes (4). It has been reported that GFJ consumption may alter the disposition of certain concomitantly administered drug metabolism probe agents (10).

GFJ contains bergamottin, a natural furanocoumarin, that can cause irreversible inhibition of intestinal enzyme CYP3A4 which metabolizes more than 60% of prescribed drugs (11-12). If a drug is metabolized at the intestinal level, its hepatic metabolism will be reduced leading to an increase in the overall systemic concentration. Depending on the drug, this increase can be of several to hundred folds (2, 13).

The inhibition of organic anion-transporting polypeptides (OATP), membrane proteins that mediate the transport of organic anions across intestinal epithelial cells, modulation of intestinal P-glycoproteins and inhibition of esterase activity are other possible mechanisms by which GFJ interacts with drugs. These mechanisms have been suggested for drugs such as ritonavir, lovastatin, fexofenadine, and talinolol (14-16).
Paracetamol (acetaminophen), an antipyretic and analgesic agent, is metabolized in the liver by conjugation with either glucuronide or sulfate moieties. Less than 15% of paracetamol undergoes metabolism by cytochrome P450 enzymes. CYP2E1 and CYP3A4 metabolize paracetamol to the metabolite N-acetyl-p-benzoquinone imine (NAPQI) (17). NAPQI is a significant alkylating agent that at usual doses undergoes detoxification by conjugation with glutathione. Therefore, paracetamol is usually regarded as a safe drug and does not cause clinically significant adverse effects if consumed within the therapeutic window. Thus, it is a widely used over-the-counter drug (18).

GFJ-paracetamol interaction has not drawn a lot of attention because of the small portion of paracetamol that undergoes cytochrome P450 enzymes metabolism. Dasgupta et al. and Samojlik et al. have shown that GFJ increased the concentrations of paracetamol in mice compared with the control after single and multiple doses, respectively (19-20). On the contrary, Qinna et al. reported a reduced bioavailability of paracetamol following multiple GFJ administration in rats and reduced salivary levels of paracetamol in human saliva following a single dose of GFJ (21-22).

Most of the previously published studies on GFJ-paracetamol interaction emphasized the pharmacokinetic part of the interaction and we could trace only one study that examined the GFJ effect on the pharmacodynamic action (i.e. analgesic) of paracetamol. Samojlik et al. only showed that GFJ, when administered with paracetamol, significantly reduced the irritant effect of acetic acid in mice; an effect attributed to the enhanced analgesic effect of paracetamol, probably to its increased bioavailability (20).

The aim of this study was, therefore, to examine some pharmacokinetic and pharmacodynamic aspects of the interaction of paracetamol with single or multiple doses of GFJ in mice.

**EXPERIMENTAL**

**Animals**
CD1 male mice (9-10 weeks old, initially weighing about 40 g) were obtained from the Small Animal House of Sultan Qaboos University. Animals were maintained on a standard pellet diet and water *ad libitum* and housed in a room with a temperature of 22 ± 2°C, relative humidity of about 60%, and with a 12 h light/dark cycle (lights on at 6:00). All procedures involving animals and their care were carried out in accordance with international laws and policies and with ethical clearance from Sultan Qaboos University Animal Ethics Committee.

**Pharmacokinetic study**
Following an acclimatization period of one week, mice (*n* = 162) were randomly distributed into three main groups (*n* = 54). The 1st group was administered a single dose of paracetamol (400 mg/kg) via gastric gavage and served as the control. The 2nd group was treated similarly to the 1st group but was also given a single dose of GFJ (10 mL/kg) one hour prior to administration of paracetamol. The 3rd group was given 10 mL/kg grapefruit juice for five consecutive days and on the 5th day, one hour after GFJ administration was also administered a single dose of paracetamol (400 mg/kg). Paracetamol and GFJ doses were selected to bracket what has been reported in the literature.

Each of the three groups was then divided into nine subgroups (*n* = 6). Each subgroup was anesthetized intraperitoneally with ketamine (100 mg/kg) and xylazine (20 mg/kg) at different time intervals as follows: 10, 20, 30, and 40 min, and 1, 2, 4, 6, and 8 h.

Table 1. The effect of treatment of mice with single or multiple doses of grapefruit juice (GFJ, 10 mg/kg) on plasma concentration of paracetamol (400 mg/kg) at several time intervals.

<table>
<thead>
<tr>
<th>Treatment Description</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
<th>40 min</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol (400 mg/kg)</td>
<td>0.88 ± 0.23</td>
<td>0.79 ± 0.12</td>
<td>0.54 ± 0.07</td>
<td>0.46 ± 0.06</td>
<td>0.23 ± 0.04</td>
<td>0.14 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Paracetamol + single dose of GFJ (10 mL/kg)</td>
<td>1.16 ± 0.07*</td>
<td>1.14 ± 0.07*</td>
<td>0.90 ± 0.09**</td>
<td>0.60 ± 0.06</td>
<td>0.56 ± 0.07**</td>
<td>0.16 ± 0.01</td>
<td>0.06 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Paracetamol + multiple doses of GFJ (10 mL/kg, 5 days)</td>
<td>0.95 ± 0.06*</td>
<td>0.88 ± 0.06</td>
<td>0.78 ± 0.09</td>
<td>0.54 ± 0.06</td>
<td>0.48 ± 0.06†</td>
<td>0.13 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

Each value in the table represents the mean ± SEM (*n* = 6 for each group). *denotes significance of Group 2 vs. Group 1 at a given time interval: where *p < 0.05, **p < 0.001. † denotes significance of Group 3 vs. Group 1 at a given time interval: where †p < 0.05.
The effect of a single or multiple doses of grapefruit juice on some pharmacokinetic parameters of paracetamol (400 mg/kg).

Blood samples (1.5 mL) from an abdominal vein were collected in plain tubes wetted with 4% sodium citrate and centrifuged at 900 g for 15 min. The obtained plasma was stored at -80°C pending analysis. Paracetamol was analyzed using a COBAS® automated analyzer (Roche Diagnostics, USA).

The pharmacokinetic parameters evaluated, C<sub>max</sub> (the maximum plasma concentration) and t<sub>max</sub> (the time to reach C<sub>max</sub>), were determined as the highest observed values in individual plasma-concentration profiles. Terminal half-life (t<sub>1/2</sub>) was derived as ln2/K, where K is the elimination rate constant. The area under the plasma concentration curve (AUC) extrapolated to 8 h (AUC<sub>0-8</sub>) was calculated as AUC(0-8) = AUC<sub>t</sub> + Ct/K, where AUC<sub>t</sub> is the area under the curve from time zero to the time of the last blood sample (8 h), and Ct is the plasma concentration at the corresponding time, calculated with use of the regression equation for estimation of the elimination rate constant. The total clearance (CL) was calculated as CL = Dose/AUC, and apparent volume of distribution was calculated as V<sub>d</sub> = CL/K. Mean residence time (MRT) was calculated as MRT = AUMC/AUC, where AUMC is the area under the first moment curve. All calculations were carried out using the linear trapezoidal method.

### Hot plate and abdominal constriction (Writhing) test

Following an acclimatization period of one week, mice (n = 18) were randomly distributed into three main groups (n = 6). The first group was given water and served as the control group. The 2<sup>nd</sup> group was administered a single dose of paracetamol (400 mg/kg) via gastric gavage and the 3<sup>rd</sup> group was treated similarly to the 2<sup>nd</sup> group but was also given a single dose of GFJ (10 mL/kg) one hour prior to administration of paracetamol.

The hot plate method was used as described before (23). Each mouse was placed on a hot plate (Ugo Basile, SRL, Varese, Italy) set at 55°C after one hour of the treatments. The time that was required for jumping or paw licking was recorded. The cut-off time of response was taken as 15 seconds to minimize damage to the mouse paws.

### Table 2. The effect of treatment of mice with single or multiple doses of grapefruit juice (GFJ, 10 mg/kg) on some pharmacokinetic parameters of paracetamol (400 mg/kg).

<table>
<thead>
<tr>
<th>Parameter/Group</th>
<th>Paracetamol</th>
<th>Paracetamol + single GFJ dose</th>
<th>Paracetamol + multiple GFJ doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the plasma concentration-time curve&lt;sub&gt;0-24&lt;/sub&gt;, AUC (mmol.min/L)</td>
<td>54.90 ± 4.23</td>
<td>81.50 ± 2.26***</td>
<td>68.98 ± 3.65†</td>
</tr>
<tr>
<td>Area under the first moment curve&lt;sub&gt;0-24&lt;/sub&gt;, AUMC (mmol.min&lt;sub&gt;2&lt;/sub&gt;/L)</td>
<td>6296.04 ± 440.53</td>
<td>6621.94 ± 195.37</td>
<td>6527.49 ± 774.73</td>
</tr>
<tr>
<td>Mean residence time, MRT (min)</td>
<td>95.93 ± 4.72</td>
<td>76.47 ± 2.25*</td>
<td>85.63 ± 6.37</td>
</tr>
<tr>
<td>Half-life, t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>67.60 ± 3.45</td>
<td>54.35 ± 1.80**</td>
<td>56.87 ± 2.43†</td>
</tr>
<tr>
<td>Plasma peak concentration, C&lt;sub&gt;max&lt;/sub&gt; (mmol/L)</td>
<td>1.06 ± 0.20</td>
<td>1.24 ± 0.07</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>Time to reach peak concentration, T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>16.67 ± 2.11</td>
<td>14.00 ± 2.00</td>
<td>14.00 ± 2.00</td>
</tr>
<tr>
<td>Apparent clearance, CL (mg/mmol.min/L)</td>
<td>0.25 ± 0.02</td>
<td>0.18 ± 0.00**</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Apparent volume of distribution, V&lt;sub&gt;d&lt;/sub&gt; (mg/mmol.min/L/min)</td>
<td>29.59 ± 4.82</td>
<td>15.43 ± 1.17*</td>
<td>18.85 ± 1.25</td>
</tr>
</tbody>
</table>

Each value in the table represents the mean ± SEM (n = 6 for each group). *denotes significance of Group 2 vs. Group 1 for a given parameter: where *p < 0.05, **p < 0.001, ***p < 0.0001. †denotes significance of Group 3 vs. Group 1 for a given parameter: where †p < 0.05
The abdominal constriction test was performed as previously described (24-25). After an hour of treatment with paracetamol and GFJ, mice were injected intraperitoneally with 10 mL/kg of 0.6% acetic acid. The total numbers of muscle constrictions were counted for 15 min.

Drugs, kits and chemicals
Paracetamol was gifted by National Pharmaceutical Industries (Muscat, Oman). Fresh grapefruits were purchased from the local market and squeezed to obtain the juice at the time of the experiment. The juice was centrifuged at 900 g for 10 min at 4°C to obtain the clarified grapefruit juice. All other chemicals used were of analytical reagent grade.

Statistical analysis
The results are expressed as the mean ± standard error of measurement (SEM). Student’s two-tailed test was used to compare the groups, and a p-value < 0.05 was considered statistically significant. GraphPad Prism version 5.01 software (GraphPad Software, Inc., San Diego, CA, USA) was used for data analysis.

RESULTS
Pharmacokinetic study
Table 1 shows the paracetamol plasma concentration at different time points. Table 2 depicts the various calculated paracetamol pharmacokinetics parameters. Both single (81.50 ± 2.26 vs 54.90 ± 4.23 mmol.min/L) and multiple doses (68.98 ± 3.65 vs 54.90 ± 4.23 mmol.min/L) of GFJ increased paracetamol concentrations, but this was only significant at 20 min, 30 min and 1 h for a single dose. The single dose of GFJ appeared to have more effect than multiple doses (68.98 ± 3.65 vs 68.98 ± 3.65 mmol.min/L). GFJ at a single dose increased the $C_{\text{max}}$ (1.24 ± 0.07 vs 1.06 ± 0.20 mmol/L) and decreased $T_{\text{max}}$ (14.00 ± 2.00 vs 16.67 ± 2.11 min), while multiple GFJ doses reduced $C_{\text{max}}$ (1.04 ± 0.05 vs 1.06 ± 0.20 mmol/L) and decreased $T_{\text{max}}$ (14.00 ± 2.00 vs 16.67 ± 2.11 min), although none of these effects was statistically significant. GFJ at a single dose significantly decreased MRT (76.47 ± 2.25 vs 95.93 ± 4.72 min), $t_{1/2}$ (54.35 ± 1.80 vs 67.60 ± 3.45 min), CL (0.18 ± 0.00 vs 0.25 ± 0.02 mg/mmol.min/L) and $V_d$ (15.43 ± 1.17 vs 29.59 ± 4.82 mg/mmol.min/L/min) and increased AUC$_{0-8}$ (81.50 ± 2.26 vs 54.90 ± 4.23 mmol.min/L), while multiple doses of GFJ exerted a similar effect, but this was not statistically significant.

Hot plate test
Figure 1 shows the time spent on the hot plate of the separate groups. The group that was given GFJ had a significantly longer reaction time on the hot plate when compared with the control group (p < 0.001), and it was also significantly longer when compared with the group given paracetamol alone (p < 0.05).
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Abdominal constriction (writhing) test

Figure 2 shows that the group given a single dose of GFJ had a slight but significant reduction in abdominal constriction due to the irritant effect of acetic acid when compared with those that were given paracetamol alone.

DISCUSSION

In this study, ingestion of GFJ increased the plasma concentration of paracetamol in mice after a single dose and to a lesser extent following multiple doses. This increase in plasma concentration of paracetamol has been reported previously by Dasgupta et al. (19). In their study, they showed that the plasma concentration and half-life of paracetamol increased at 1 and 2 hours following the ingestion of 200 microliters of white GFJ, but there were no differences in the elimination half-life. In comparison with their study, we sampled paracetamol at 9-time points (10 to 480 min), which provided enough and perhaps a more reliable pool of data to extract and compare various paracetamol pharmacokinetic parameters. We found that MRT, \( t_{1/2} \), \( T_{\text{max}} \), CL and \( V_d \) were reduced by GFJ, while AUC\(_{0-4h}\) and \( C_{\text{max}} \) increased. These findings are in line with the observed increase in paracetamol plasma concentrations. However, contrary to our present results and those of Dasgupta et al., Qinna et al. found that frequent administration of GFJ reduced the oral bioavailability of paracetamol in rats (19-21). This discrepancy might be related to species differences.

It has been demonstrated that mice models of paracetamol metabolism and paracetamol-induced hepatotoxicity are similar to those for humans and therefore clinically more relevant experimental models than experimental rats’ models (26). Nevertheless, further studies comparing mice and rats’ models in relation to GFJ-paracetamol interaction are warranted. In addition, determination of the concentration of paracetamol metabolites, which was not done here, might be needed to understand fully the impact of GFJ on paracetamol pharmacokinetics, although the reported pharmacokinetic data in our control are similar to those previously established (27-28).

In this study, we further provided evidence that the increased paracetamol concentration induced by GFJ is accompanied by an increase in one of its main pharmacodynamics actions (viz antinociception). This was ascertained using the classical hot plate and writhing tests. GFJ-treated mice showed significantly longer reaction time on the hot plate and significantly less abdominal constriction than in mice given paracetamol alone. Both results indicate an increase in the antinociceptive effect of paracetamol, which is in line with the increase in the drug’s plasma concentrations. Our finding that GFJ significantly reduced the irritant effect of injected acetic acid in paracetamol-treated mice is in agreement with the previously reported data of Samojlik et al. (20).

Irreversible inhibition of intestinal cytochrome P450 enzymes is the main mechanism by which GFJ alters a drug’s disposition (8-9). CYP3A4, the major
isoform expressed in the intestinal mucosa, is highly susceptible to interaction with GFJ. It is also the enzyme that metabolizes almost 60% of all prescribed drugs, and hence several have been reported to interact with GFJ (11-12). Other isoforms that have been shown to be affected by GFJ, but to a lesser degree, are CYP2C9, CYP2C19 and CYP2D6 (14). Paracetamol is a known substrate for CYP3A4, though this pathway only accounts for less than 15% of its metabolism (17). This might explain the small alteration observed in paracetamol pharmacokinetic parameters compared with the several folds seen with drugs that are highly metabolized by CYP3A4 isoforms (2, 13).

Other mechanisms involving GFJ-drug interaction include inhibition of esterase activity, modulation of intestinal P-glycoprotein and interfering with OATP (29). We are not sure if, and to what extent, these mechanisms play a role in the GFJ-paracetamol interaction. Alteration to intestinal P-glycoprotein has been shown to increase the bioavailability of some drugs such as indinavir (30). It has also been reported that drugs that are transported by P-glycoproteins are also metabolized by CYP3A4 (31). OATP inhibition by GFJ might cause a reduction in the systemic concentration of drugs, as seen with aliskiren (32). However, the mechanism involving esterase inhibition is unlikely to be applicable to paracetamol as it is not a prodrug. The effects of these mechanisms on paracetamol systemic concentration and their clinical relevance require further studies and exploration.

CONCLUSION

GFJ ingestion altered the pharmacokinetics and pharmacodynamics of paracetamol in mice. The implications of these changes in humans and their clinical relevance need to be further investigated.

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Authors’ contribution

MZ and BA were responsible for study concept, designs and participated in writing the manuscript. AH collected and analyzed the data. MZ performed data analysis. BA supervised the study. All authors read and approved the final manuscript.

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