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## Co-administration of fresh grape fruit juice (GFJ) and bergamottin prevented paracetamol induced hepatotoxicity after paracetamol overdose in rats



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### ABSTRACT

The aim of this study was to evaluate small doses of known cytochrome P450 enzyme inhibitors, grapefruit juice (GFJ) and one of its components, bergamottin (BGT), for the prevention of paracetamol (PAR)-induced hepatotoxicity after overdose in rats. Six groups of 15 Sprague Dawley (SD) rats each were treated with single oral doses of either saline, PAR only 1725 mg/kg, PAR + GFJ low dose (2 ml) and PAR + GFJ high dose (3 ml), PAR + BGT 0.05 mg/kg (BGT-low) and PAR + BGT 0.22 mg/kg (BGT-high). Thereafter, 5 rats from each group were sacrificed after 24, 48 and 72 h and, on each occasion, blood samples were collected for determination of liver and renal function, full blood count (FBC) and PAR concentration. A piece of liver was sent for histopathology. By 48 h the liver enzymes in the PAR-only group were significantly ( $P < 0.05$ ) higher than in the PAR + GFJ and PAR + BGT groups, i.e., alanine transaminase (ALT)  $837 \pm 268$  u/L and aspartate transaminase (AST)  $1359 \pm 405$  for PAR only; versus ALT  $34 \pm 48.8$  u/L and AST  $238 \pm 221$  for PAR + GFJ-high; ALT  $22 \pm 13.9$  and AST  $168 \pm 49.6$  for PAR + BGT-high; and ALT  $52 \pm 7.2$  u/L and AST  $147 \pm 153$  for the control group. The results correlated with the histopathology findings where livers of the PAR-only group exhibited severe centrilobular and hepatocyte necrosis. In conclusion, GFJ and BGT prevented PAR-induced hepatotoxicity after PAR overdose in rats, and this calls for appropriate observation studies in humans.

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### 1. Introduction

PAR is a widely used analgesic and antipyretic agent. While it is generally safe at recommended doses, acute overdose of PAR can cause fatal liver damage [1,2]. After PAR overdose, the major metabolic pathways of glucuronidation and sulphation become saturated and the excess PAR is bioactivated by cytochrome P450

isoenzymes to a hepatotoxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) [3]. Currently, N-acetylcysteine (NAC) is the drug of choice for treating PAR overdose. It converts the NAPQI to non-toxic cysteine and mercaptate conjugates. Unfortunately, the use of NAC is hampered by uncertainty on outcome in relation to the time and route of administration, the fact NAC does not stop the production of NAPQI and the fear for hypersensitivity reactions. On the other hand, despite the understanding that some cytochrome P450 isoforms are responsible for activation of PAR to NAPQI, the use of enzyme inhibitors for prevention and/or treatment of PAR-induced hepatotoxicity is still not well researched. In a previous report by Walubo and co-workers [4], it was demonstrated that

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administration of a hepatotoxic dose of PAR in combination with known inhibitors of cytochrome P450 3A4, 2E1 and 1A2, *i.e.*, ketoconazole, isoniazid and caffeine, prevented the development of PAR-induced hepatotoxicity. Unfortunately, because of their side effects and therapeutic use, these drugs could not be investigated further for treatment or prevention of PAR-induced hepatotoxicity. As such, there was a need to search for appropriate candidates for use in the prevention PAR hepatotoxicity.

Although GFJ interaction with cytochrome P450 had been known for some time, it was only recently that furanocoumarins were identified as the mediators of the interaction between GFJ and CYP3A4 [5,6]. Generally furanocoumarins are well known plant phytotoxins believed to serve as the plants' defense mechanism against predators. However, some furanocoumarin-monomers found in the GFJ such as BGT (GF-I-2), 6',7'-dihydroxybergamottin (DHB), and dimers such as paradisine A (GF-I-1) and paradisine B (GF-I-4) are suitable for human consumption [7,8]. In this perspective, along with the wide availability of GFJ, it was found more attractive to evaluate GFJ or extract for prevention of PAR hepatotoxicity. Therefore, the aim of the study was to evaluate GFJ and one of its component furanocoumarins, BGT, for the prevention of PAR-induced hepatotoxicity after overdose in rats.

## 2. Materials and methods

### 2.1. Materials and apparatuses

PAR syrup and tablets were purchased from a local pharmacy; pure acetaminophen powder and BGT were purchased from Sigma–Aldrich™ (St. Louis, MO, USA), while grapefruits (Star Ruby) were bought from a local super market. The rounded end needles (16 G-3", curved 3.00 mm ball) used for oral gavage feeding were purchased from Popper & Sons, New York. The human cytochrome P450 (Bacculosomes®, Invitrogen™, Carlsbad, CA, USA) were used to screen GFJ extract for enzyme inhibition.

### 2.2. Preliminary experiments

#### 2.2.1. Preparation of grapefruit extracts

Fifty (50) Star Ruby grapefruits were peeled, and the peels were homogenised for 2 min using a food blender, during which 1000 ml of distilled water was added. The homogenate was filtered to remove the pulp, after which it was centrifuged for 15 min at  $11,963 \times g$  (10,000 r.p.m.). The supernatant was extracted with 2.5 volumes of ethyl acetate, shaken vigorously and centrifuged for 15 min. The aqueous phase was removed and the organic layer evaporated using a rotary evaporator at 45 °C. The residue (or oily peel extract) was weighed, reconstituted with water, and stored at –20 °C until analysis. The juice was squeezed from the grapefruit by hand, after which it was filtered and centrifuged for 5 min at  $7026 \times g$  (13,400 r.p.m.) and stored at 4 °C until analysis. Of note, furanocoumarins content in juices stored for up to 20 weeks at 4 °C remained fairly constant [9].

#### 2.2.2. HPLC assay

The two grapefruit products were characterised by qualitative HPLC screening for furanocoumarins using BGT as the standard. 100 µl of the peel-extract was centrifuged at  $7026 \times g$  (13,400 r.p.m.) for 5 min and 20 µl of the supernatant was injected into the HPLC. The juice was also centrifuged at  $7026 \times g$  (13,400 r.p.m.) for 5 min and the supernatant (100 µl) was directly injected into the HPLC. BGT stock solution in acetonitrile was diluted with the mobile phase to 100 µg/ml.

The HPLC system was a Hewlett Packard model 1100 that was equipped with an autosampler (Waldbronn, Germany), and UV detector (Shimadzu, Tokyo, Japan). The mobile phase comprised of Solvent A and B. Solvent A consisted of 10% acetonitrile in TEAP buffer, whereas Solvent B was 0.01% sulphuric acid in acetonitrile. TEAP buffer was prepared by mixing phosphoric acid (2.9 g) with 15.54 g of tetraethylammoniumhydroxide (TEAH) in 500 ml distilled water. The chromatographic conditions consisted of a Phenomenex® C<sub>18</sub> column (150 mm × 4.60 mm, 3 µm particle size), coupled to a SecurityGuard™ C<sub>18</sub> (4 mm × 3 mm) guard column (Phenomenex®, Torrance, CA, USA), with a mobile phase (30% A) flow rate of 1.0 ml/min and a UV detector set at 210 nm.

Under these conditions, BGT was eluted at 18 min (Fig. 1a), and this retention time was similar to a peak in the GFJ at 17 min (Fig. 2a) and UV spectrum (Figs. 1b and 2b). Although the peel extract exhibited many peaks, there was no significant peak between 14 and 20 min. Therefore, the peel extract was not processed any further.

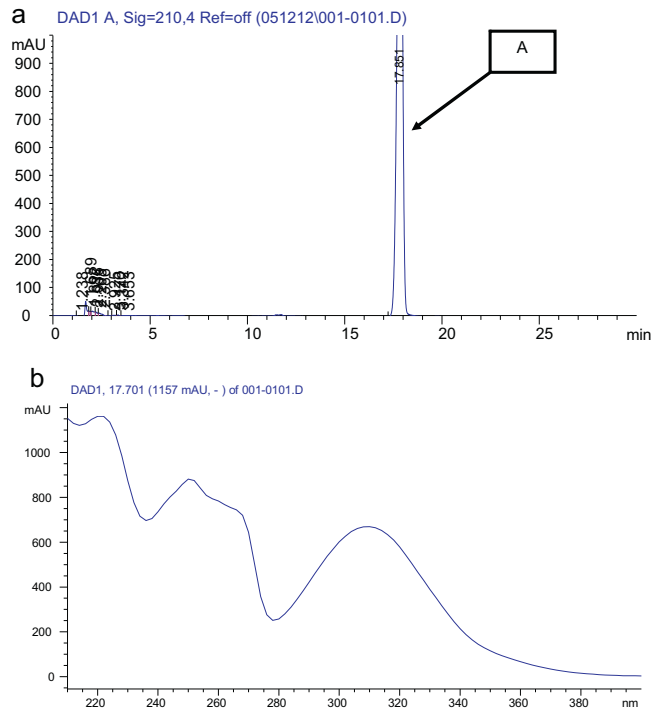
#### 2.2.3. Cytochrome P450 activity

The grapefruit juice was further evaluated to determine if it had cytochrome P450 enzyme inhibitor properties required for the product to be used in the prevention of PAR hepatotoxicity. GFJ was screened for its effect on the activities of human CYP2E1, CYP3A4 and CYP1A2 *in vitro*, the isoforms implicated in the activation of PAR to a toxic metabolite. The effect of GFJ on the activity of a specific P450-isoform was tested by observing for changes in the rate of metabolism with the addition of increasing amount (0, 5, 10, 15, 20 µl; *n*=5) of the GFJ to the reaction mixture (250 µl, with phosphate buffer pH 7.4). This was done separately for each of the following P450-isoforms: CYP3A4, CYP1A2, and CYP2E1. The rate of metabolism for CYP3A4 was determined by monitoring for the rate of 4-hydroxylation of midazolam (0.25 mM), while for CYP2E1 was by 4-hydroxylation chlorzoxazone (2 mM), and CYP1A2 by *O*-dealkylation of 7-ethoxyresorufin (0.1 mM). GFJ inhibited the activity of each enzyme with increasing concentration of GFJ (Fig. 3).

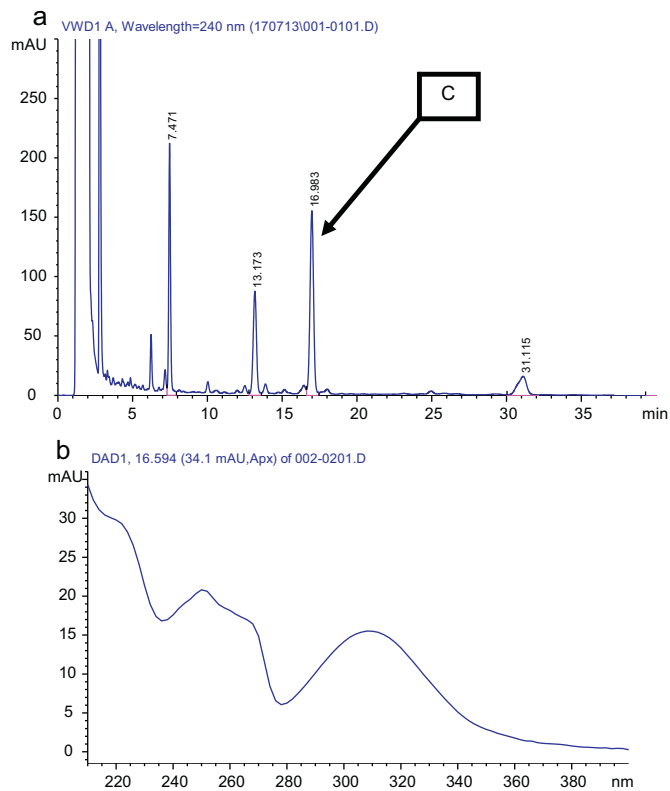
### 2.3. The main experiment

#### 2.3.1. Animal care

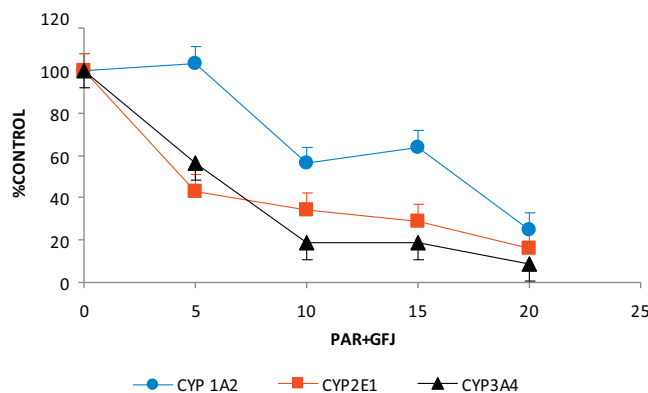
The study was approved by the Animal Ethics Committee of the University of the Free State (NR 10/2012). Sprague–Dawley (SD) rats weighing between 200 and 250 g were used. Animals were kept and treated at the Animal House of the University. Here they were fed and looked



**Fig. 1.** (a) A chromatogram of bergamottin (Peak A) in standard in mobile phase. (b) A UV spectrum of Peak A (bergamottin).



**Fig. 2.** (a) A chromatogram of grapefruit juice (un-extracted) with several peaks. Peak C has similar retention time to bergamottin (Fig. 1a). (b) A chromatogram of the UV spectrum of peak C in grapefruit juice is similar to that of bergamottin (Fig. 1b).



**Fig. 3.** The effect of increasing concentration of GFJ on the activity of human CYP1A2, CYP2E1 and CYP3A4 *in vitro* [PAR was added to mimic the clinical situation where GFJ will be used in an environment of high PAR concentrations,  $n = 5$ ].

after by qualified staff and their cages cleaned once a week. Standard rat chow and water was available *ad libitum*. All animals were inspected for any abnormal signs every day.

### 2.3.2. Determination of the toxic dose of PAR

A preliminary experiment to determine a dose of PAR that would induce hepatotoxicity was conducted. Hepatotoxicity was diagnosed when the liver enzymes, ALT increased above 5-fold that of the control animal (note; animal care was as described later). Three groups of 9 SD rats (200–250 g) each were treated with a single dose of either 1000, 1725 or 1822 mg/kg of PAR and, from each group, 3 rats were sacrificed after 24, 48 and 72 h after dosing. One group of 3 rats was not treated. Blood was tested for FBC, LFT and RFT.

There was no change in liver enzymes on the three occasions after 1000 mg dose. At 48 h, elevated liver enzymes were observed after 1725 mg/kg dose (ALT 837 (647–1026), AST 1359 (1073–1645) and ALP 296 (191–401)), and after 1822 mg/kg dose (ALT 449 (64–728), AST 1201 (156–1651) and ALP 231 (153–364)). Whereas after 72 h the liver enzymes in the 1725 mg/kg group remained high (ALT  $419.0 \pm 586$ , AST  $512.0 \pm 593$ , ALP  $260.0 \pm 36$ ), they fell far lower in the 1822 mg/kg group (ALT  $44.3 \pm 3$ , AST  $104.0 \pm 14$ , ALP  $223.7 \pm 82$ ) most probably due to cell death. Since the 1725 mg/kg group exhibited hepatotoxicity that was sustained over the study period, it was selected for use in the subsequent experiments.

### 2.3.3. Experimental design

Rats were divided into five groups of 15 animals each and were administered orally with either 1 ml of saline (Saline) or 2 ml GFJ (GFJ-low) or 3 ml GFJ (GFJ-high) or 0.05 mg/kg BGT (BGT-low) or 0.22 mg/kg BGT (BGT-high), and five rats from each group were sacrificed after 24, 48 and 72 h. Another five groups of 15 animals each were also administered orally but with either 1725 mg/kg PAR (PAR) or 1725 mg/kg PAR and 2 ml GFJ (PAR+GFJ-low) or 1725 mg/kg PAR and 3 ml GFJ (PAR+GFJ-high) or 1725 mg/kg PAR and 0.05 mg/kg BGT (PAR+BGT-low) or 1725 mg/kg PAR and 0.22 mg/kg BGT (PAR+BGT-high), and five rats from each group were sacrificed after 24, 48 and 72 h. Except where indicated, the respective doses of BGT

and PAR were dissolved in 1 ml of saline as ordered by the animal ethics committee.

### 2.3.4. Blood collection and surgical procedure

Under isoflurane anaesthesia, blood (5 ml) was drawn by cardiac puncture and immediately aliquoted to the appropriate test tubes. Thereafter, the abdomen was opened through a vertical incision to expose the liver. A piece of liver (10 g) was cut and stored in 10% formalin and sent for histopathology. The remainder of the liver was quickly dissected out, washed in a 1.5% potassium chloride solution, frozen with liquid nitrogen and stored at  $-85^{\circ}\text{C}$ . Thereafter, the animals were sacrificed by exsanguination whilst still under anaesthesia. PAR concentrations were measured in our laboratory using a validated HPLC method. The full blood count, liver and renal function tests were done at the National Health Laboratory System (NHLS; Bloemfontein, South Africa), while histopathology of the livers was performed and reported by an independent veterinary pathologist (Idexx Laboratories, Johannesburg, South Africa).

### 2.3.5. PAR HPLC assay

PAR concentrations were measured in rat plasma by HPLC. It involved protein precipitation of 50  $\mu\text{l}$  of rat plasma with 30  $\mu\text{l}$  2.5% zinc sulphate after addition the internal standard (4-aminoacetophenone), followed by centrifugation. The supernatant was directly injected into the HPLC. The HPLC system was an Agilent, Hewlett Packard 1100 series, equipped with a 1260 Infinity quaternary pump (Waldbronn, Germany) with a 1260 Infinity degasser attached to a G1313A autosampler (Waldbronn, Germany), and a G1314A UV wavelength detector (Tokyo, Japan). Separation of PAR and 4-aminoacetophenone was achieved by running the mobile phase at a flow rate of 1 ml/min. over a Phenomenex<sup>®</sup> C<sub>18</sub> (4.60 mm  $\times$  250 mm) 5  $\mu\text{m}$  analytical column, coupled to a Phenomenex<sup>®</sup> SecurityGuard<sup>™</sup> C<sub>18</sub> (4 mm  $\times$  3 mm) guard column (Torrance, CA, USA). Compounds were detected by UV at a wavelength of 240 nm. The mobile phase consisted of 0.01% trifluoroacetic acid in distilled water (solvent A) and 100% HPLC grade acetonitrile (solvent B), and was run isocratically (80:20, v/v). Under these conditions, PAR was eluted at 4.25 min and

IS at 6.25 min. The method was linear ( $y = 0.603x + 0.089$ ;  $r^2 = 0.9957$ ;  $n = 5$ ) and the limit of detection was  $0.1 \mu\text{g/ml}$ .

### 2.3.6. Statistical analysis

Data was analysed by non-parametric methods using the GraphPad Instat statistical programme and the Mann–Whitney Test was used for data comparison with the level of significance set at  $P < 0.05$ .

## 3. Results

Daily animal observations did not reveal any abnormal clinical signs. The FBC, liver and renal functions tests for the groups treated with GFJ and BGT alone were normal at all doses (not shown), implying that these agents did not cause hepatotoxicity at the respective doses.

Table 1 shows the changes in liver function tests over the 72 h following administration of PAR. There was a significant increase in the liver function tests (ALT and AST) in the rats that were treated with PAR-only by 24 and 48 h. The increase was more than 5 times those of the control group (saline) which indicated that the PAR-only group had experienced hepatotoxicity. When PAR was co-administered with grapefruit juice, remarkably low liver enzyme levels (ALT and AST) were observed at 48 and 72 h. Similarly, co-administration of PAR with BGT prevented the increase in liver enzymes after 48 and 72 h. Of note, GFJ and BGT co-administration were associated with high PAR concentrations at 24 h followed by a rapid drop thereafter (Fig. 4).

During surgery, direct observation of the organs, the livers of the rats that were treated with PAR only (PAR-only) were highly discoloured with some exhibiting goose bump like spots which were most severe after 48 h of treatment, and still present after 72 h. The livers and kidneys of rats treated with PAR+GFJ and PAR+BGT were normal at all doses with a few showing a slight spotting and discolouration after 48 h, which had recovered after 72 h. Fig. 5 shows the representative sections of the livers with the respective histopathology reports. PAR alone caused severe hepatotoxicity that was marked with severe centrilobular and hepatocyte necrosis at 48 and 72 h (Fig. 5 slides B & C), while co-administration with GFJ and BGT prevented this to occur (Fig. 5 slides E & F, and slides H & I, respectively). As expected from clinical observations, PAR hepatotoxicity was not evident at 24 h.

Table 2 illustrates the changes in the full blood count after 24, 48 and 72 h of drug administration. In all test animals, PAR was associated with thrombocytopenia (low platelet count) that was moderate in the groups treated with GFJ and BGT. The rest of the full blood count parameters were not affected.

### Pathology reports

Fig. 5 shows sections of a rat livers from groups treated with: single toxic dose of PAR-only (A, B, C); PAR+GFJ (D, E, F); and PAR+BGT (G, H, I) as follows:

- A: 24 h after dosing with PAR.
  - The section of liver reveals mild granular vacuolar degeneration and very mild swelling of the hepatocytes. The trabecular structure can still be visualised, as

can the sinusoids, although slightly reduced in diameter. There is a mild mononuclear portal inflammatory infiltrate mainly composed of macrophages.

- B: 48 h after dosing with PAR.
  - Under low power ( $4\times$  magnification) severe centrilobular bridging necrosis can be seen. Large confluent areas of necrosis characterised by loss of hepatocyte nuclei, disarrangement of the cords and loss of structure is present in the centrilobular areas, with extension from the centrilobular regions through the zones to link up with other centrilobular areas. The hepatocytes are lightly eosinophilic and there is loss of the trabecular structure and the acute necrosis is associated with mild haemorrhage as well as acute inflammatory cells including polymorphonuclear cells and mononuclear cells. The rest of the hepatocytes between the bridging necrosis reveal mild to moderate, granular, vacuolar degeneration and mild fatty change.
- C: 72 h after dosing with PAR.
  - Severe centrilobular bridging necrosis with severe centrilobular and periportal inflammation characterised by mononuclear inflammatory cells. The bridging necrosis is similar to that already described, characterised by eosinophilia of the hepatocytes with loss of nuclei and trabecular structure. Increased mitotic figures at approximately four mitoses per high power field ( $40\times$  magnification) can be seen close to the necrotic zones, but within the preserved areas. Some of the necrotic areas maintain an outline of the hepatocytes, suggesting acute coagulative necrosis. There is a mild leukostasis.
- D: 24 h after dosing with PAR+GFJ (3 ml):
  - Mild vacuolar change and swelling, mild leukostasis and reduced numbers of mitoses. Mild portal mononuclear inflammatory infiltration.
- E: 48 h after dosing with PAR+GFJ (3 ml):
  - Mild to moderate vacuolar change and degeneration with mild, portal, mononuclear inflammatory infiltrates and very few mitotic figures.
- F: 72 h after dosing with PAR+GFJ (3 ml):
  - with few mitotic figures and mononuclear inflammatory infiltration.
- G: 24 h after dosing with PAR+BGT (0.22 mg/kg):
  - Moderate to severe vacuolar degeneration and severe swelling of the hepatocytes with a mild mononuclear portal cell infiltration.
- H: 48 h after dosing with PAR+BGT (0.22 mg/kg):
  - Moderate vacuolar degeneration and mild mononuclear portal infiltration with few mitoses.
- I: 72 h after dosing with PAR+BGT (0.22 mg/kg):
  - Severe vacuolar degeneration and swelling of the hepatocytes with loss of trabecular structure and odd, small granules of bile pigment could be seen within the hepatocytes and sinusoids.

## 4. Discussion

This study has illustrated that GFJ and BGT can protect against PAR-induced hepatotoxicity during PAR overdose in rats and in a dose dependent manner. The mechanism is most probably due to prevention of the metabolic

**Table 1**The liver function tests (mean  $\pm$  SD) and paracetamol plasma concentrations (median & range) during the 72 h.

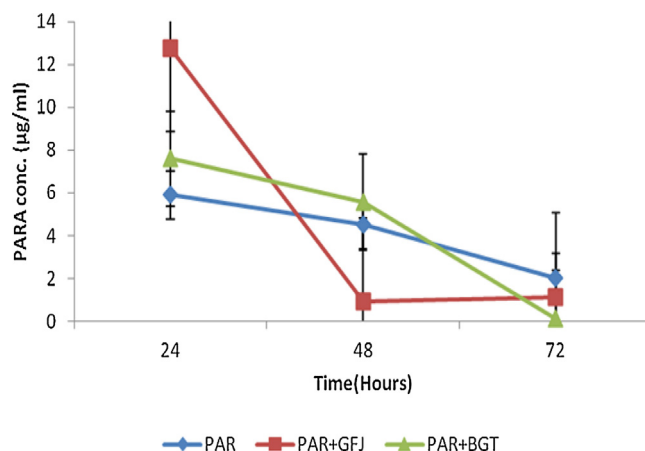
Group (n = 5)	Weight before Rx	Liver function tests (units/l)			PARA conc. ( $\mu$ g/ml)
		ALP	ALT	AST	
<b>SAL (control)</b>					
24 h	223 $\pm$ 6.9	226 $\pm$ 45.2	44 $\pm$ 10.6	60 $\pm$ 10.6	NA
48 h	217 $\pm$ 12.7	366 $\pm$ 11.2	52 $\pm$ 7.2	147 $\pm$ 153.3	NA
72 h	214 $\pm$ 16.5	136 $\pm$ 88.7	39 $\pm$ 0.7	54 $\pm$ 2.8	NA
<b>PAR-only</b>					
24 h	242 $\pm$ 1.6	319 $\pm$ 2.1	63 $\pm$ 41.3	156 $\pm$ 76.2	5.72 (4.38–28.15)
48 h	223 $\pm$ 4.1	<b>296 <math>\pm</math> 148.5</b>	<b>837 <math>\pm</math> 268.0</b>	<b>1359 <math>\pm</math> 404.5</b>	4.45 (2.23–6.67)
72 h	234 $\pm$ 11.0	267 $\pm$ 38.9	419 $\pm$ 690.1	512 $\pm$ 657.9	2.02 (1.61–15.34)
<b>PAR + GFJ low</b>					
24 h	242 $\pm$ 6.4	62 $\pm$ 64.7	9 $\pm$ 2.5	69 $\pm$ 5.0	7.31 (6.18–10.05)
48 h	206 $\pm$ 6.5	312 $\pm$ 72.3	628 $\pm$ 764.5	641 $\pm$ 660.7	3.72 (2.07–4.01)
72 h	204 $\pm$ 3.5	285 $\pm$ 102.9	5 $\pm$ 2.8	85 $\pm$ 43.8	0.13 (0.00–0.14)
<b>PAR + GFJ high</b>					
24 h	227 $\pm$ 12.9	212 $\pm$ 114.1	18 $\pm$ 7.6	141 $\pm$ 65.8	<b>12.87</b> (3.99–18.81)
48 h	207 $\pm$ 6.5	222 $\pm$ 34.0	<b>34 <math>\pm</math> 48.8</b>	238 $\pm$ 220.5	0.92 (0.92–1.42)
72 h	215 $\pm$ 18.0	327 $\pm$ 34.6	27 $\pm$ 26.1	147 $\pm$ 52.4	1.14 (0.99–1.67)
<b>PAR + BGT low</b>					
24 h	208 $\pm$ 5.1	194 $\pm$ 86.0	9 $\pm$ 3.0	94 $\pm$ 45.6	4.90 (3.77–11.28)
48 h	221 $\pm$ 7.2	156 $\pm$ 104.6	<b>17 <math>\pm</math> 13.1</b>	81 $\pm$ 7.0	0.84 (0.49–2.43)
72 h	205 $\pm$ 1.5	283 $\pm$ 74.8	12 $\pm$ 7.1	83 $\pm$ 18.6	0.84 (0.49–2.43)
<b>PAR + BGT high</b>					
24 h	207 $\pm$ 7.5	268 $\pm$ 48.4	9 $\pm$ 4.4	104 $\pm$ 9.2	<b>7.78</b> (6.84–8.66)
48 h	212 $\pm$ 4.6	314 $\pm$ 36.8	<b>22 <math>\pm</math> 13.9</b>	168 $\pm$ 49.6	5.57 (5.26–16.93)
72 h	218 $\pm$ 8.1	237 $\pm$ 18.6	12 $\pm$ 7.6	110 $\pm$ 38.2	0.13 (0.00–0.16)

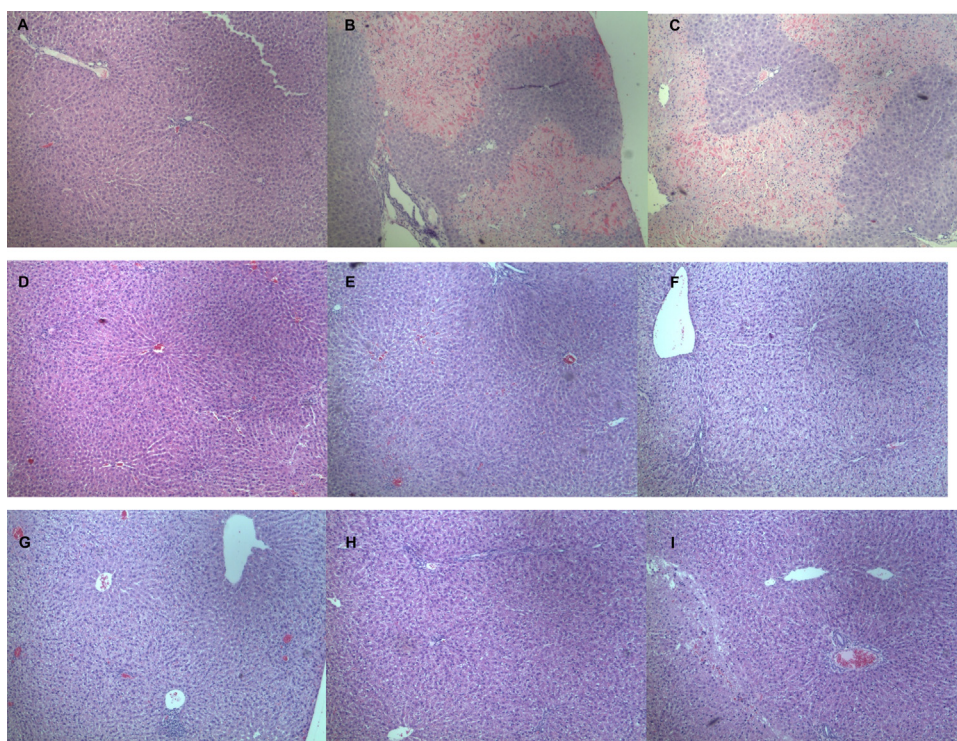
Rx = treatment; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; PAR = paracetamol; SAL = saline; GFJ = grapefruit juice; BGT = bergamottin.

activation of PAR by inhibition of cytochrome P450. BGT was selected for use in this study because it was commercially available and, is considered safe to use in humans, as indicated by their use in several human drug-disposition studies where they were given at dosages up to 35  $\mu$ mol [8,10]. Indeed, several compounds in the GFJ were demonstrated to be potent inhibitors of P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, and the enzyme inhibition was related to total furanocoumarin content whereby no single furanocoumarin could be marked as the sole cause [7,11]. Moreover, the furanocoumarin dimers, paradisine A (GF-I-1) and paradisine B (GF-I-4), were more potent enzyme inhibitors than the monomers, BGT (GF-I-2; over 100 times) and 6-7-dihydroxybergamottin (DHB;

over 20 times) [7]. In general, further characterisation of the GFJ for furanocoumarins would not be conclusive given that more new furanocoumarins have been identified in GFJ and their standards are not yet available to aid their analysis [12]. Therefore, for this study, it was sufficient to establish that the GFJ used had enzyme inhibitor properties for the three CYP450 isoforms implicated in the metabolic activation of PAR. A faster drop in PAR concentrations in the GFJ and BGT groups than in the PAR-only group was most probably due to a better functioning of their livers (metabolism and/anti-oxidant) in these groups, compared to the damaged liver of the PAR-only group.

Because of wide interspecies variations in sensitivity to PAR hepatotoxicity, whereby rats in particular are more

**Fig. 4.** Paracetamol plasma concentrations in the PAR-only, PAR + GFJ and PAR + BGT groups at 24, 48 and 72 h.



**Fig. 5.** Representative histopathology slides (4×) of rat livers at different times since dosing with the respective drugs (A = 4 h, B = 48 h and C = 72 h after dosing with PAR; D = 24 h, E = 48 h and F = 72 h after dosing with PAR + GFJ; and G = 24 h, H = 48 h and I = 72 h after dosing with PAR + BGT). See the text for the corresponding pathology reports.

**Table 2**

Haematology results (full blood count; mean ± SD) during the 72 h after drug administration.

Group (n = 3)	Full blood count							
	WCC	RCC	Hb	Hct	MCV	MCH	MCHC	Plt
SAL (control)								
24 h	4.8 ± 0	6.2 ± 0	13.3 ± 0	0.388 ± 0	62.2 ± 2	21.1 ± 0	33.9 ± 1	970 ± 129
48 h	6.0 ± 0	6.3 ± 0	12.9 ± 1	0.387 ± 0	61.8 ± 1	20.6 ± 0	33.4 ± 1	1023 ± 162
72 h	4.9 ± 1	6.5 ± 0	13.0 ± 0	0.392 ± 0	60.5 ± 0	20.1 ± 0	33.3 ± 1	957 ± 88
PAR-only								
24 h	4.1 ± 1	7.6 ± 0	15.2 ± 0	0.439 ± 0	57.6 ± 1	19.9 ± 0	34.5 ± 0	1159 ± 81
48 h	5.9 ± 0	7.5 ± 0	15.3 ± 1	0.464 ± 0	61.1 ± 1	20.4 ± 0	33.1 ± 0	<b>310 ± 305</b>
72 h	4.7 ± 1	6.8 ± 1	13.6 ± 2	0.406 ± 0	58.9 ± 4	19.8 ± 1	33.7 ± 1	326 ± 293
PAR + GFJ low								
24 h	6.4 ± 1	6.7 ± 0	13.4 ± 1	0.397 ± 0	59.6 ± 1	20.0 ± 1	33.6 ± 0	1101 ± 172
48 h	5.1 ± 1	6.1 ± 1	12.8 ± 3	0.387 ± 0	63.4 ± 1	20.9 ± 0	33.0 ± 0	<b>333 ± 78</b>
72 h	6.1 ± 1	6.2 ± 1	12.4 ± 1	0.389 ± 0	62.3 ± 1	19.8 ± 0	31.9 ± 0	627 ± 59
PAR + GFJ high								
24 h	6.2 ± 2	6.6 ± 0	13.7 ± 1	0.405 ± 0	61.0 ± 1	20.6 ± 0	33.7 ± 0	1088 ± 85
48 h	4.7 ± 1	6.1 ± 0	13.0 ± 1	0.395 ± 0	64.5 ± 1	21.2 ± 1	33.0 ± 0	<b>464 ± 74</b>
72 h	6.7 ± 0	6.6 ± 0	13.1 ± 0	0.399 ± 0	64.9 ± 2	21.3 ± 2	32.8 ± 0	492 ± 163
PAR + BGT low								
24 h	4.5 ± 0	5.8 ± 0	11.6 ± 1	0.353 ± 0	61.0 ± 3	20.1 ± 1	33.0 ± 0	944 ± 185
48 h	6.5 ± 0	6.4 ± 1	13.5 ± 2	0.420 ± 0	65.6 ± 2	21.0 ± 0	32.0 ± 0	<b>587 ± 134</b>
72 h	5.4 ± 1	6.2 ± 1	12.8 ± 1	0.380 ± 0	61.6 ± 0	20.7 ± 0	33.6 ± 0	1065 ± 122
PAR + BGT high								
24 h	7.0 ± 1	5.9 ± 1	12.4 ± 2	0.382 ± 0	64.4 ± 1	20.8 ± 1	30.6 ± 1	923 ± 68
48 h	6.6 ± 1	6.4 ± 1	13.5 ± 1	0.392 ± 0	61.7 ± 3	21.1 ± 1	34.2 ± 1	<b>773 ± 25</b>
72 h	5.6 ± 1	6.7 ± 1	13.5 ± 1	0.415 ± 0	62.3 ± 3	20.3 ± 1	32.6 ± 0	780 ± 119

PAR = paracetamol; GFJ = grapefruit juice; BGT = bergamottin; WCC = white cell count; RCC = red cell count, Hb = Haemoglobin; Hct = Haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; Plt = platelets.

resistant to PAR toxicity, instead of using doses from previous studies, a toxic dose of PAR for this study was first established before the subsequent testing. Nevertheless, histopathological lesions of PAR hepatotoxicity (centrilobular necrosis) were similar to those reported in other species including humans.

The essence of this study was to illustrate that co-administration of a small dose of an enzyme inhibitor such as GFJ and BGT with a toxic dose of PAR can prevent development of PAR-induced hepatotoxicity in rats, thereby opening avenues for further evaluation of these compounds in this regard. Since most patients with PAR overdose almost always arrive at hospital long after the ingestion, it was envisaged that the best way to prevent development of the hepatotoxicity is by ensuring that patients take the PAR together with the antidote (enzyme inhibitor). This can be achieved by addition of a small quantity of the enzyme-inhibitor to each PAR tablet. At normal doses, the small quantity of the enzyme-inhibitor in each tablet would not inhibit or interfere with enzyme activity, but when a patient takes an overdose or many tablets that are potentially hepatotoxic, the total amount of the enzyme-inhibitor would be high enough to inhibit cytochrome P450 enzymes and prevent the hepatotoxicity. Specifically, given that the hepatotoxic dose of PAR in humans is 100 mg/kg, a 70 kg man would need 15 tablets of 500 mg PAR each to induce hepatotoxicity. This patient would require 15.4 mg of BGT (0.22 mg/kg) to prevent development of PAR hepatotoxicity. It would therefore be plausible to impregnate each PAR tablet with at least 1 mg of BGT.

As indicated earlier, PAR is useful drug, yet it is a common cause of drug-induced hepatotoxicity [1,2]. Currently, owing to the fear of NAC-induced hypersensitivity reactions, NAC is only recommended for patients who have ingested a potentially hepatotoxic dose of PAR. Unfortunately, the actual PAR dose or number of tablets taken is not known in the majority of patients. Worse still, in the clinical setting, clinical hepatotoxicity is only detectable after 24–48 h when liver enzymes are high, and this, together with the fact that NAC is most useful when given in first 8 h, makes management of PAR overdose difficult [13]. The use of a normogram not only requires PAR concentration measurements which are not available in most clinical settings, but also makes treatment more expensive. In this study, fresh GFJ was preferred for testing because it is readily available and would not need prescriptions for administration. Using GFJ would not only obviate all the problems with NAC enumerated above, but also reduce morbidity, mortality and save money. The exact quantities cannot be deduced from these animal studies, but even a low concentration of GFJ (2.5% solution of GFJ) was shown to inhibit P450 enzyme activity by 60% [11]. Since toxicology studies

may not be possible in humans, there is a need for further investigations in other animals and clinical observations in humans to enable appropriate clinical application.

In conclusion, the results of this study demonstrate that GFJ and BGT can prevent PAR-induced hepatotoxicity.

### Conflict of interest

None.

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