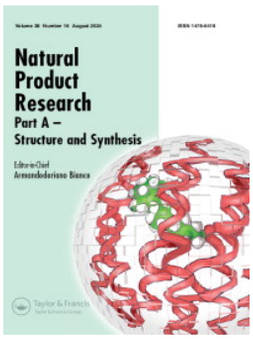

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# Alleviation of favipiravir-induced hepatotoxicity using hepatoprotective herbal extracts in wistar rats

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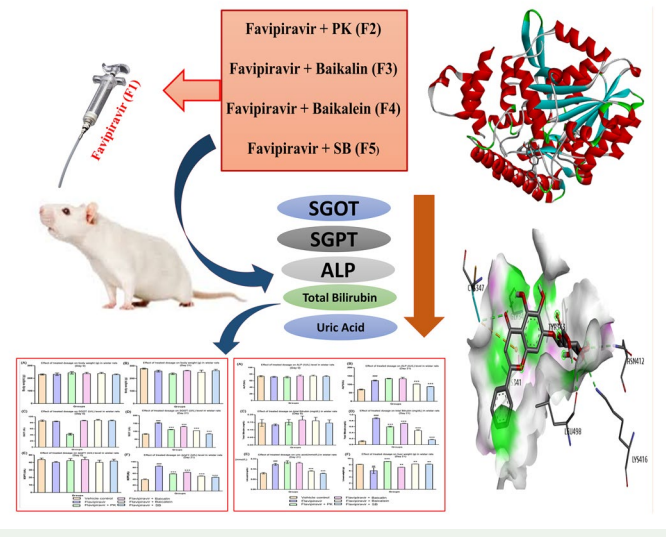
**ABSTRACT**

Teratogenicity and hyperuricaemia are the main side effects of favipiravir, an antiviral drug recently found its use to treat mild to moderate coronavirus (COVID-19) infections. This study investigated the beneficial effect of herbal extracts like *Picrorrhiza kurroa* (PK) and *Scutellaria baicalensis* (SB) and their active chemical constituents (baicalin and baicalein) on favipiravir-induced hepatotoxicity in rats. The formulation combinations included favipiravir, favipiravir + PK extract, favipiravir + pure baicalin, favipiravir + pure baicalein, and favipiravir + SB extract designated as F1, F2, F3, F4 and F5 respectively that were administered to rats orally for 21 days. Favipiravir caused increased levels of SGOT, SGPT, ALP, total bilirubin, and uric acid and decreased liver weight which was alleviated when alloherbal formulation of favipiravir and baicalein combination and favipiravir and SB extract was used. This paper highlights an attractive proposition to ameliorate favipiravir-induced hepatotoxicity using hepatoprotective agents.

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## 1. Introduction

COVID-19 is an infectious disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Favipiravir, an antiviral drug, was approved for the new and re-emerging pandemic influenza in Japan in 2014 and showed potent *in vitro* activity against SARS-CoV-2. As a viral RNA polymerase inhibitor, favipiravir has been extensively investigated and shows significant promise in the treatment of a wide range of RNA virus infections through clinical studies (Łagocka et al. 2021 and Joshi et al. 2021). An additional human hexokinase inhibiting potential of favipiravir has been demonstrated recently (Kulkarni and Padmanabhan 2022).

Teratogenicity and hyperuricaemia have been reported as the main side effects of favipiravir and a recent case of cholestatic liver injury (Yamazaki et al. 2021) with elevated levels of liver enzymes such as AST (SGOT), ALT (SGPT), and ALP is reported (Bayram et al. 2021 and Woolbright and Jaeschke 2012). SARS-CoV-2 enters the host cell through the ACE-2 receptor which is expressed in various organs, including the lungs, heart, and liver with the liver being the potential target for SARS-CoV-2 infection. Herbal extracts such as *Picrorrhiza kurroa* (PK) and *Scutellaria baicalensis* (SB) have been reported for their hepatoprotective action (Misar Wajpeyi 2020) due to its key components baicalin and baicalein, (Ding et al. 2019, Kim et al. 2007 and Kumar et al. 2017 and Zhao et al. 2006). Both these agents protect against several types of liver diseases including viral hepatitis, xenobiotic induced liver injury, and cholestatic liver injury (Almeleebia et al. 2022).

To alleviate the effect of favipiravir on the liver, we included herbal extracts such as PK and SB along with favipiravir and also tablets of favipiravir with pure baicalin, and baicalein and investigated the effect of these formulations on favipiravir-induced hepatotoxicity in rats.

## 2. Results and discussion

### *2.1. Extraction of P. kurroa and Scutellaria baicalensis and composition of the formulations*

The percentage yield of the extract was calculated by estimating amount of extract obtained per 100 g of the raw material. Table S1 details the compositions of all the formulations used in this study.

### *2.2. Pre-compression parameters*

#### *2.2.1. Angle of repose (θ) and bulk density*

The angles of repose for all three formulations are shown in Table S2. The angles were found to be in the range of 36–40, indicating that all formulations had fair flow characteristics (Saifullah et al. 2016). The bulk density of a material represents the mass-to-volume (including interparticle void volume) ratio of an untapped powder. Table S2 shows the results for both loose bulk density and tapped density. The results are within the acceptable range, with no significant variation between loose bulk density and tapped density.

#### 2.2.2. Percentage compressibility and Hausner's ratio

The results for the percent compressibility are shown in Table S2. The percent compressibility for all three formulations lies within the range of 16–20, indicating that the compressibility is fair for all the formulations tested. All of the formulations shown in Table S2 have a Hausner's ratio that is within the permissible range between 1.19 and 1.25.

### 2.3. Post-compression parameters

Table S3 shows the post-compression parameters such as weight, hardness, thickness, and disintegration time (DT) in minutes. All the formulations were found to be within the acceptable range for the parameters tested.

#### 2.3.1. Hardness test and tablet thickness

Table S3 shows the results of the hardness test performed using the Monsanto hardness tester. The reduced standard deviation suggested that the hardness of all the formulations was consistent and they had high mechanical strength and hardness. The thickness of the tablets was found to be in the range of $2.5 \pm 0.035$ mm to $2.38 \pm 0.04$ mm respectively (Table S3).

#### 2.3.2. Weight variation test

The results of the Table S3 show the % weight variation for all of the formulations. All the tablets passed the weight variation test since the percent variation was less than 10%, which is the pharmacopeia's limit. The variation was found to be between 60 and 62 mg. All the tablets had the same weight. This is owing to the fair flow properties and compressibility of the compositions.

#### 2.3.3. Disintegration time

The disintegration time for all formulations is shown in Table S3. The disintegration time for all formulations was less than 2 min, which indicates that all the formulations were of the immediate release dosage form.

#### 2.3.4. Content of the drug by HPLC

The content of favipiravir in the tablets of all the formulations was assayed by HPLC was found to be as claimed in the label (Table S3).

### 2.4. Molecular docking

The docking study was performed on ALT (SGPT) (3IHJ; 2.30 Å) and AST (SGOT) (3II0; 2.05 Å) and the structures were obtained from the protein data bank (PDB) (Ayyamperumal et al. 2021). The binding energy (Kcal/mole) of Baicalein and Baicalin were analysed by the molecular modelling software (AutoDock 4.2.6), and the output interaction of docking was showed (2D plot) by Accelrys Discovery Studio

Visualiser, which displayed the hydrophobic interactions of ligand with active site amino acids in the selected proteins (Table S4; Figures S1 and S2). Closer view of Figure S1 and S2 infers that the ligands interacted with numerous amino acids within the hydrophobic cavity of both ALT (SGPT) and AST (SGOT), as well as along interacted with various bond interactions such as hydrogen, and other bonds (alkyl, π-alkyl, and Van der Waals).

Through docking studies of SGPT with baicalein and baicalin, baicalein showed better binding than baicalin ((−7.16 Kcal/mol vs. −6.87 Kcal/mol) (Table S4) and almost similar binding energies of −6.40 and −6.26 Kcal/mol, for SGOT respectively. Based on the molecular docking and *in vivo* findings, it may be summarised that both baicalein and baicalin exhibited therapeutic agents towards the healing of hepatic injuries induced by favipiravir, in this case.

## 2.5. Biochemical parameters

### 2.5.1. Effect of favipiravir on the body weight (g) of animals

There was no difference in the body weight of the treated groups on days 0 and 21 in comparison to control animals (Figure 1).

### 2.5.2. Effect of favipiravir on SGPT and SGOT in rats

Administration of favipiravir (F1) for 21 days caused a significant ($p < 0.001$) increase in the SGOT (153.5 ± 2.55 U/L) and SGPT (85.00 ± 1.34 U/L) levels as compared to vehicle control (SGOT: 83.83 ± 2.15 U/L; SGPT: 39.17 ± 2.41 U/L). Treatment with favipiravir + PK (F2), favipiravir + baicalin (F3), favipiravir + baicalein (F4), and favipiravir + SB (F5) extract for 21 days, caused a significant ($p < 0.001$) decrease in the SGOT (F2: 112.5 ± 1.33 U/L; F3: 129.5 ± 2.32 U/L; F4: 105.0 ± 0.316 U/L; and F5: 83.82 ± 2.15 U/L) and SGPT (F2: 58.83 ± 0.79 U/L; F3: 64.33 ± 0.80 U/L; F4: 51.20 ± 0.92 U/L; and F5: 47.33 ± 0.66 U/L) levels when compared to favipiravir (Figure 1). The combination of favipiravir + SB (F5) having more activity towards the balancing of SGOT and SGPT levels to normal.

### 2.5.3. Effect of favipiravir on ALP levels

Administration of favipiravir (F1) for 21 days caused a significant ($p < 0.001$) increase in the ALP levels (F1: 123.5 ± 2.79 IU/L) as compare to vehicle control (70.17 ± 2.762 IU/L). Treatment with favipiravir + baicalein (F4: 102.2 ± 0.96 U/L) and favipiravir + SB (F5: 87.50 ± 1.64) extract for 21 days, caused a significant ($p < 0.001$) decrease in the ALP levels when compared to favipiravir (Figure 2). However, treatment with favipiravir + PK and favipiravir + baicalin did not cause any significant change in the ALP levels, but in case of favipiravir + baicalein (F4) and favipiravir + SB (F5) shown considerable effect to stabilise the level of ALP.

### 2.5.4. Effect of favipiravir on total bilirubin levels

Administration of favipiravir (F1) for 21 days caused a significant ($p < 0.001$) increase in the total bilirubin levels (0.87 ± 0.01 mg/dl) as compare to vehicle control (0.12 ± 0.01 mg/dl). Treatment with favipiravir + PK (F2: 0.60 ± 0.01 mg/dl), favipiravir + baicalin (F3: 0.70 ± 0.01 mg/dl), favipiravir + baicalein (F4: 0.48 ± 0 0.03 mg/dl), and

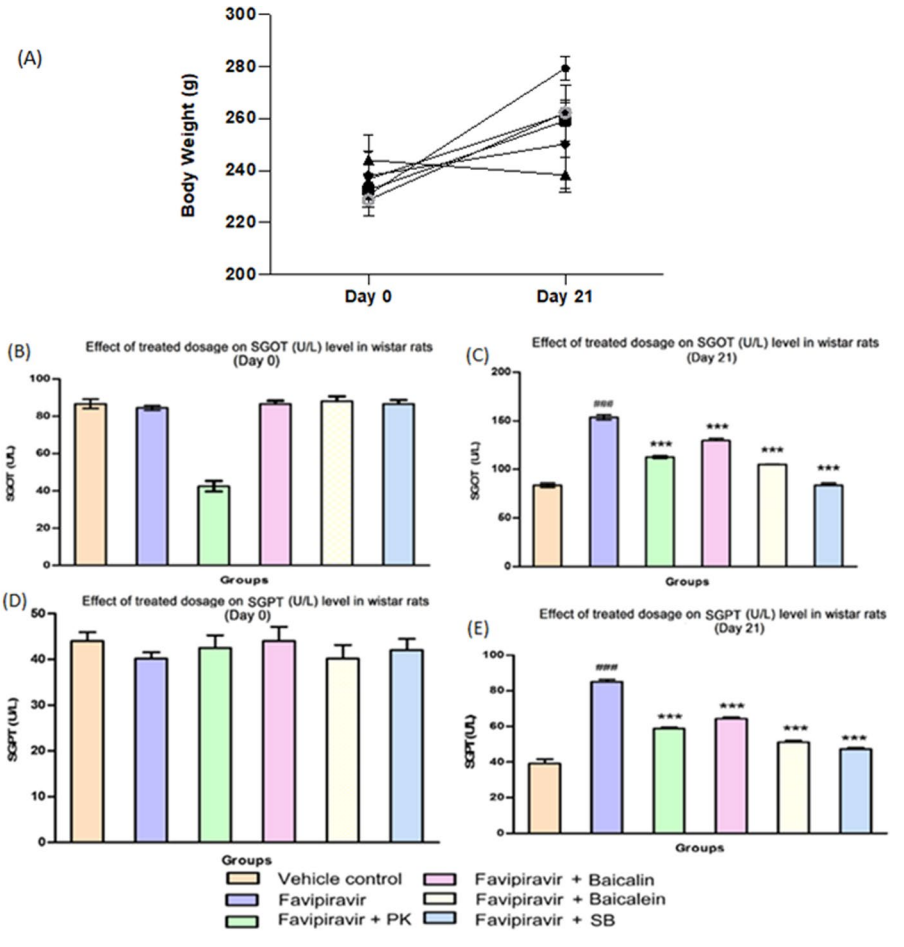


**Figure 1.** Effect of treated dosage on body weight, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) in wistar rats on day 0 and day 21. Panel A shows body weight of all the rats on day 0 and day 21 where the labels are given as —●— control animals; —▼— favipiravir treated animals; —■— favipiravir + *P. kurroa* extract treated animals; —◆— favipiravir + baicalin treated animals; —▲— favipiravir + baicalein treated animals; —⊕— favipiravir + *S. baicalensis* extract treated animals. Panels B and C shows the level of SGOT and panels D and E show SGPT level variations on day 0 and day 21. Statistical analysis values were expressed as mean ± SEM ($n = 6$). One way ANOVA followed by Dunnett's test with ##$p < 0.01$, ###$p < 0.001$ when compared to vehicle control; and **$p < 0.01$, ***$p < 0.001$ when compared to all treated groups.

favipiravir + SB (F5: 0.16 ± 0.01 mg/dl) extract for 21 days, caused a significant ($p < 0.001$) decrease in the total bilirubin levels when compared to favipiravir (Figure 2). Besides all treated groups, favipiravir + baicalein (F4) and favipiravir + SB (F5) shown similar result as compared to the vehicle control group.

### *2.5.5. Effect of favipiravir on serum uric acid levels*

Administration of favipiravir (F1) caused a significant ($p < 0.001$) increase in serum uric acid level (0.12 ± 0.005 mmol/L) when compared to the vehicle control group (0.08 ± 0.003 mmol/L). Treatment with favipiravir + baicalein (F4: 0.09 ± 0.002 mmol/L)

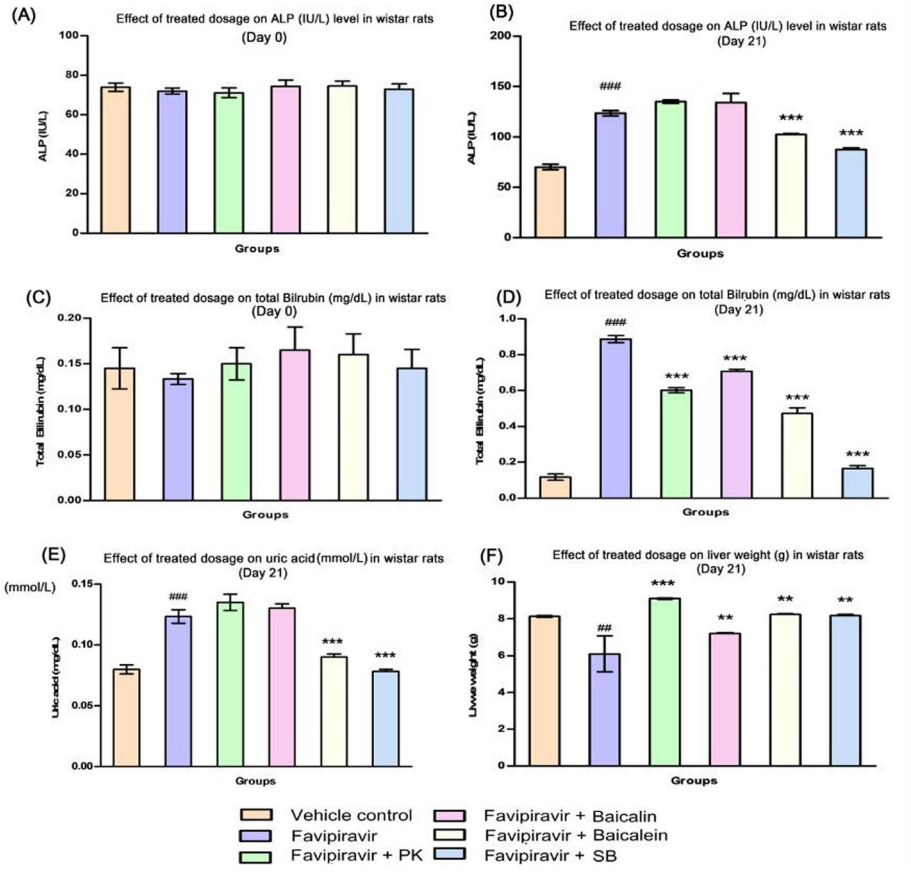


**Figure 2.** Effect of treated dosage on alkaline phosphatase (ALP; day 0 and 21), total bilirubin level (day 0 and 21), uric acid (day 21), and liver weight (day 21) in wistar rats. Panels A and B represents the level of ALP of treated rat groups on 0th and 21st day while panels C and D represents the total bilirubin level in treated rats on 0th and 21st day. Panels E and F show the uric acid level, liver weight of different treated rat groups on 21st day. Statistical analysis values were expressed as mean ± SEM ($n = 6$). One way ANOVA followed by Dunnett's test with ##$p < 0.01$, ###$p < 0.001$ when compared to vehicle control; and **$p < 0.01$, ***$p < 0.001$ when compared to all treated groups.

and favipiravir + SB (F5: 0.08 ± 0.001 mmol/L) extract for 21 days, caused a significant ($p < 0.001$) decrease in the serum uric acid levels when compared to favipiravir (Figure 2). However, treatment with favipiravir + PK (F2) and favipiravir + baicalin did not cause any significant change in serum uric acid levels.

### *2.5.6. Effect of favipiravir on liver weight (g)*

Administration of favipiravir (F1) caused a significant decrease (6.10 ± 0.98 g) in liver weight (g) when compared to the vehicle control ($p < 0.01$; 8.13 ± 0.05 g) group. Treatment with favipiravir + PK (F2: 9.10 ± 0.34 g), favipiravir + baicalein (F4: 8.23 ± 0.05 g), and favipiravir + SB (F5: 8.16 ± 0.07 g) extract for 21 days, caused a significant increase in the liver weight when compared to favipiravir (Figure 2) ($p < 0.01$). However, treatment with favipiravir + baicalin (F3: 7.20 ± 0.05) did not cause any significant change in the liver weight.

To assess the degree of liver injury, the levels of liver enzymes such as SGOT, SGPT, and ALP, apart from total bilirubin and serum uric acid were determined. Our results suggested that administration of baicalein and SB in rats resulted in substantial reduction in the levels of SGOT, SGPT, ALP, total bilirubin, uric acid, and liver weight. The liver weight loss caused by favipiravir was restored by baicalein and SB. The outcome of the biochemical estimations of liver markers fortifies the outcome of the molecular docking analysis of baicalein and Baicalin against the protein ALT (SGPT) and AST (SGOT).

The dose of Favipiravir given to animals of this study is 8 mg/rat/day for 21 days which equates to 40 mg/kg/day. We did not see any major change in the liver histology in animals of favipiravir treated groups of our study (Figure S6) and this observation is supported by similar observations of Bilici et al. (2023) where the authors observed merely mild edema in liver of animals given 100 mg favipiravir/kg and severe edema, lymphocyte infiltration and hydropic degeneration in the favipiravir 400 mg/kg (FAV-400) group.

The 3 C-like protease, also known as C30 endopeptidase, is the main protease found in SARS-CoV-2 and is essential for viral replication. The ethanolic extract of *S. baicalensis* that contains baicalin and baicalein, inhibits SARS-CoV-2 3 C-like protease activity with an $IC_{50}$ value of 0.39 µM for baicalein and 83.4 µM for baicalin (Li et al. 2021; Liu et al. 2021). Similar to this result, we obtained a significant decrease in liver enzymes and an increase in the liver weight due to treatment with favipiravir + baicalein (F4).

## 3. Conclusions

*S. baicalensis*, used to treat hepatitis, hepatic fibrosis, and cancer of the liver, has shown to successfully reduce fibrosis and lipid peroxidation in several experiments (Zhao et al. 2006). Baicalin, the major flavonoid component extracted from SB, has been shown to have excellent therapeutic benefits in liver ailments. Our results support the observations of Bisht and Ram (2017) that combined allopathy with herbal drug to reduce the dose and side effects of the allopathic drugs. Interestingly enough, both baicalein and baicalin has been reported to have significant antiviral activity against SARS-CoV-2, which makes them attractive agents for addressing the liver toxicity issues due to favipiravir and also independently would target the multiplication of the COVID-2 virus, the causative agent of COVID-19.

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## Authorship contribution statement

Rohan Magdum: Performed experiments and generated the data. Sanman Kolhe: Arrived at the concept, performed experiments, and interpreted the data. Pramod Kolsure: Prepared formulations and generated the data. Umesh Chandra Dash: Prepared preliminary draft of the manuscript, reviewed and interpreted the data. Sriram Padmanabhan: Ideated the concept, designed, supervised the study, edited and reviewed the manuscript.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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