นิพนธ์ต้นฉบับ

Effects of *Andrographis paniculata* and Favipiravir Combination and Monotherapies on Liver Biochemistry in COVID-19 Patients: A Retrospective Cohort Study

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Abstract

Introduction and Objective: This study addresses the National Clinical Practice Guideline (CPG) that did not recommend using a combination of favipiravir (FV) and *Andrographis paniculata* (Burm. f.) Nees (AP, or *Fa Thalai Chon* in Thai) crude drug (144 mg of andrographolide per day) in the treatment for COVID-19 due to potential serious side effects. The objective is to assess the safety profile of this combination compared to individual drug use, specifically focusing on liver biochemistry. Additionally, the study aims to identify factors influencing liver function in patients after FV and/or AP usage.

Methods: The retrospective design involved retrieving medical records of COVID-19 patients who were admitted to Chao Phya Abhaibhubejhr Hospital between January 1 and December 31, 2021, and who received either FV, according to the recommended dose by the CPG for 5–10 days thereafter, or AP, with a dose of 4.8 g of standardized powder (equivalent to 144 mg of andrographolide) per day for 5 days, or a combination of both. The monitoring was undertaken to identify abnormalities in patients' liver enzymes, namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB), by identifying deviations of 2–3 times beyond the upper limit of normal (ULN).

Results: Following the inclusion and exclusion criteria, the records of 564 cases were analyzed. In the assessment of post-treatment liver enzyme elevation at least 2 times ULN, AP groups (prescribed to younger, healthy patients) showed no elevations in all four enzymes, and no group displayed increased ALP or TB levels. However, 0.87% in the FV+AP group had AST levels exceeding 3 times ULN, while 1.06% and 2.17% in the FV and FV+AP groups showed ALT levels over 3 times ULN. One case of drug-induced liver injury (DILI) was observed in both FV and FV+AP groups. No significant factors increasing abnormal liver enzyme likelihood were identified.

Discussion: Some of the findings could support safety concern regarding the use of combined FV and AP. However, it should be noted that patients receiving FV and FV+AP may exhibit greater disease severity, so may those with higher doses of FV medication, underlying diseases, advanced age, and other concurrent medications. Therefore, it is difficult to conclusively determine whether liver abnormalities are solely attributable to the FV or FV+AP medication.

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Conclusions and Recommendations: The study indicates that AP at a dose of 144 mg/day showed a promising safety profile for liver function, suggesting its suitability for young and healthy patients. AP should also be considered safe for common cold and influenza, with a dosage 3 times lower than that for COVID-19. However, 1.06% and 2.17% of patients in the FV and FV+AP groups had ALT levels exceeding 3 times, and 0.87% in the FV+AP group had AST levels exceeding 3 times ULN, indicating susceptibility to drug-induced liver injury (DILI). Therefore, careful monitoring of liver function is warranted following the administration of FV and FV+AP, especially in older patients, those with underlying diseases, and those taking concomitant medications metabolized in the liver.

Key words: *Andrographis paniculata*, favipiravir, COVID-19, liver biochemistry

การศึกษาผลของฟ้าทะลายโจรอย่างเดียวและใช้ร่วมกับยาฟาวิพิราเวียร์ต่อค่าชีวเคมีของตับ ในการรักษาผู้ป่วยโรคติดเชื้อไวรัสโคโรนา 2019 (COVID-19): การศึกษาย้อนหลัง

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บทคัดย่อ

บทนำและวัตถุประสงค์: แนวทางเวชปฏิบัติการรักษาโรคติดเชื้อไวรัสโคโรนา 2019 (COVID-19) ระดับประเทศไม่แนะนำให้ใช้ยาฟาวิพิราเวียร์ (FV) ร่วมกับฟ้าทะลายโจร (AP) เนื่องจากอาจมีผลข้างเคียงที่ร้ายแรง การศึกษานี้มีวัตถุประสงค์เพื่อติดตามความปลอดภัยของการใช้ยาทั้งสองชนิดที่ใช้ร่วมกัน เมื่อเปรียบเทียบกับการใช้เป็นยาเดี่ยว นอกจากนี้ยังมีการหาปัจจัยที่มีผลต่อการทำงานของตับหลังจากใช้ยา FV และ/หรือ AP

วิธีการศึกษา: การศึกษาย้อนหลัง โดยใช้ข้อมูลจากเวชระเบียนของผู้ป่วย COVID-19 ที่เข้ารับการรักษาในโรงพยาบาลเจ้าพระยาอภัยภูเบศร ระหว่างวันที่ 1 มกราคม - 31 ธันวาคม 2564 และได้รับยา FV ครั้งละ 1,800 มิลลิกรัม วันละ 2 ครั้ง ในวันแรก และได้ยาครั้งละ 800 มิลลิกรัม วันละ 2 ครั้ง ในวันถัดมาจนถึง 5-10 วัน, AP ขนาดยาผงมาตรฐาน 4.8 กรัมต่อวัน ซึ่งมีสารแอนโดรกราโฟไลด์ 144 มิลลิกรัมต่อวัน เป็นเวลา 5 วัน หรือทั้งสองชนิด โดยมีการติดตามความผิดปกติของค่าเอนไซม์ตับ ได้แก่ aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) และ total bilirubin (TB) ที่สูงเกินเกณฑ์จากค่า upper limit of normal (ULN) 2-3 เท่า

ผลการศึกษา: เมื่อคัดกรองผู้ป่วยตามเกณฑ์การคัดเข้าและคัดออก มีข้อมูลของผู้ป่วย 564 รายที่นำมาวิเคราะห์ พบว่าผู้ป่วยที่ได้รับ AP อย่างเดียว เป็นผู้ป่วยที่มีอายุน้อย และ/หรือไม่มีโรคประจำตัว ซึ่งไม่มีผู้ป่วยรายใดในกลุ่มนี้มีค่าเอนไซม์ตับทั้ง 4 ชนิดเพิ่มขึ้นอย่างน้อย 2 เท่า และในผู้ป่วยทุกกลุ่มไม่พบค่า ALP และ TB สูง อย่างไรก็ตามพบผู้ป่วย 2 ราย (0.87%) ในกลุ่ม FV+AP มีค่า AST เพิ่มขึ้นเกิน 3 เท่า ของ ULN และพบผู้ป่วย 3 ราย (1.06%) ในกลุ่ม FV และ 5 ราย (2.17%) ในกลุ่ม FV+AP ที่มีค่า ALT เพิ่มขึ้นมากกว่า 3 เท่า ของ ULN สำหรับการเกิดการบาดเจ็บที่ตับเนื่องจากยา พบในกลุ่ม FV และ FV+AP กลุ่มละ 1 ราย ทั้งนี้ไม่พบปัจจัยที่มีผลต่อการทำงานของตับอย่างมีนัยสำคัญทางสถิติ

อภิปรายผล: ผลการศึกษาบางส่วนสามารถใช้สนับสนุนข้อห่วงใยเรื่องความปลอดภัยของการใช้ยา FV และ AP ร่วมกัน แต่มีข้อสังเกตว่าผู้ป่วยที่ได้รับ FV และ FV+AP อาจมีความรุนแรงของโรคมากกว่า รวมถึงมีการได้รับ

FV ในขนาดสูง มีโรคประจำตัว อายุมาก และได้รับยาอื่นร่วมด้วย ดังนั้น จึงยากที่จะสรุปว่าความผิดปกติของตับเกิดจากการได้รับ FV หรือ FV+AP เพียงอย่างเดียว

ข้อสรุปและข้อเสนอแนะ: การศึกษาพบว่า การให้ยา AP ในขนาดที่มีสาร andrographolide 144 มิลลิกรัม/วัน แสดงให้เห็นแนวโน้มด้านความปลอดภัยของการทำงานของตับ ในการสั่งใช้ยาในผู้ป่วยที่อายุน้อย และไม่มีโรคประจำตัว และยังสนับสนุนความปลอดภัยการใช้ยาฟ้าทะลายโจรสำหรับการรักษาโรคไข้หวัด หรือไข้หวัดใหญ่ ซึ่งมีขนาดยาที่ใช้ต่ำกว่าในการศึกษานี้ 3 เท่า อย่างไรก็ตามผู้ป่วย 1.06% และ 2.17% ในกลุ่ม FV และ FV+AP มีค่า ALT เกิน 3 เท่า ของ ULN และผู้ป่วย 0.87% มีค่า AST เกิน 3 เท่า ของ ULN และมีความเสี่ยงต่อ DILI ด้วยเหตุนี้ การใช้ FV และ FV+AP จึงควรติดตามการทำงานของตับอย่างระมัดระวัง โดยเฉพาะอย่างยิ่งในผู้ป่วยสูงอายุ ผู้ที่มีโรคประจำตัว และมีการใช้ยาที่ถูกเปลี่ยนแปลงที่ตับร่วมด้วย

คำสำคัญ: ฟ้าทะลายโจร, ฟาวิพิราเวียร์, โรคติดเชื้อไวรัสโคโรนา 2019, ชีวเคมีของตับ

Introduction

Herbal medicine (HM) plays a crucial role in Thailand's healthcare system. During the initial wave of the COVID-19 pandemic, the use of HM in Thailand gained significant attention due to the lack of readily available standard treatments. The concept of repurposing existing drugs was embraced to provide treatment options. When favipiravir (FV) was initially added to the national guidelines for COVID-19 treatment, it was recommended as an additional therapy for adult patients experiencing progression of lung infiltration[1], whereas *Andrographis paniculata* (Burm.f.) Wall. Ex Nees (AP) was added to the national guidelines for managing mild COVID-19 cases in June 2021[2] after its inclusion in the National List of Essential Herbal Medicines (NLEHM). Later, as evidence emerged demonstrating a reduction in disease severity when FV was administered early, it became a standard treatment for symptomatic COVID-19 cases, although it was recommended that doctors refrain from recommending the concurrent use of AP and FV, citing concerns about possible severe side effects[3].

Chao Phya Abhaibhubejhr Hospital (CAH) first utilized AP for adult COVID-19 cases with the admission of the first patient in March 2020. This decision was based on AP's recognized antiviral[4] and immune-boosting properties[5]. The medical staff had become accustomed to its use and its widespread availability in Thai hospitals over the previous decade[6]. Despite national guidelines recommending the early use of FV, AP continued to be administered within our hospital to asymptomatic and mild cases. Additionally, to potentially leverage synergistic effects, the combination of AP and FV was employed for

treating moderate cases. However, as mentioned above, by mid-2021, the guidelines no longer endorsed the use of AP and FV in combination. Consequently, there was a significant decline in the usage of AP within the hospital from that point onwards.

Based on previous research and the unpredictability of disease severity, the usage of AP raised concerns regarding its potential liver-related side effects. In a study conducted on HIV-infected patients, AP high dose usage resulted in a significant 109.3% increase in Alanine transaminase (ALT) levels during the third week after initiation[7]. However, enzyme levels returned to baseline after treatment cessation. In the first instance of AP use in COVID-19 patients, one out of five patients exhibited a 1.7-fold increase in ALT levels from their normal baselines on the fifth day of AP administration, at a dose of andrographolide 180 mg/day for five days[8].

A systematic meta-analysis conducted in 2020 identified the most prevalent adverse events associated with FV use included nausea, vomiting, diarrhea, chest pain, and elevated uric acid and serum liver transaminase levels[9]. Additionally, a case report highlighted that high doses of FV induced liver injury during the treatment of COVID-19[10-11]. However, the specific liver effects resulting from the combination of AP and FV remain unknown, and this situation appears to be unique to Thailand. The aim of this study is to investigate, using real world data, the safety of the combination of FV and AP as compared to either FV or AP alone with respect to the liver functions of COVID-19 patients. Additionally, the study aims to identify the factors that influenced liver function in these patients after medication use. The ultimate goal is to improve quality of herbal medicine care by making use of real-world evidence.

Methodology

Material

This retrospective study involves the use of data extracted from medical records of CAH in Prachinburi province. The study specifically focuses on patients diagnosed with COVID-19 (ICD-10 code U07.1 - COVID-19, Virus identified) who received either AP, FV, or a combination of both, and who were admitted to the hospital between January 1st and December 31st, 2021. The research was approved by the ethical committee of CAH (IRB-BHUBEJHR-179).

We included patients in the analysis who had normal liver enzyme levels before taking the medication (as per CAH's reference values shown in Table 1) and who had undergone at least one liver enzyme test after starting the

medication. Patients who received AP or FV for less than five days were not included in the analysis (as depicted in Figure 1).

Treatment

In the hospital's clinical practice, AP was prescribed for asymptomatic and mild cases, while FV+AP was prescribed for moderate cases or mild cases with high risk factors. However, the use of AP, either alone or in combination, was discontinued when the guidelines were updated in June 2021[2] to recommend using FV as early as possible, and not using the combination of these two medications at all.

For FV, the loading dose on Day 1 was 1,800 mg twice daily, followed by a maintenance dose of 800 mg twice daily, administered for 5 to 10 days thereafter. The AP, manufactured by Chao Phya Abhaibhubejhr Hospital Foundation, had a daily dosage of 4.8 gram (400 mg per capsule) of standardized powder. Patients were instructed to take 4 capsules three times daily which is equivalent to 144 mg of andrographolide per day for 5 days. The AP products used in patients did not originate from the same production batch. In cases where both FV and AP were used in combination, the original doses remained consistent with monotherapy. However, the dosage and duration of the medications could be adjusted based on the signs and symptoms exhibited by each patient.

Outcome

Abnormality of liver biochemistry, namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) was observed. In order to understand the effects of the medications on liver function, elevations of liver values of 2 or 3 times above the upper limit of normal (ULN) were identified.

The upper limits of normal for AST, ALT, ALP and TB identified by CAH were used as references, as indicated in Table 1.

Table 1 Normal values of liver biochemistry used at CAH

Liver biochemistry	Normal Values	
	Male	Female
AST (U/L)	10-50	10-35
ALT (U/L)	10-50	10-35
ALP (U/L)	40-129	35-104
TB (mg/dL)	0.0-1.2	0.0-1.2

Data Analysis

For data analysis, both descriptive and inferential statistics were utilized. Descriptive statistics encompass measures such as frequency, percentage, mean and median for continuous variables. In terms of inferential statistics, we employed methods including the chi-square test to assess relationships between categorical variables, One-Way Analysis of Variance (ANOVA), and the Kruskal-Wallis Test for comparing means among multiple independent groups. Furthermore, Binary Logistic Regression Analysis was used to identify the factors that predict an increase in liver biochemistry among the three groups (FV, AP, and FV+AP). All statistical analyses were conducted using STATA version 16.1 software.

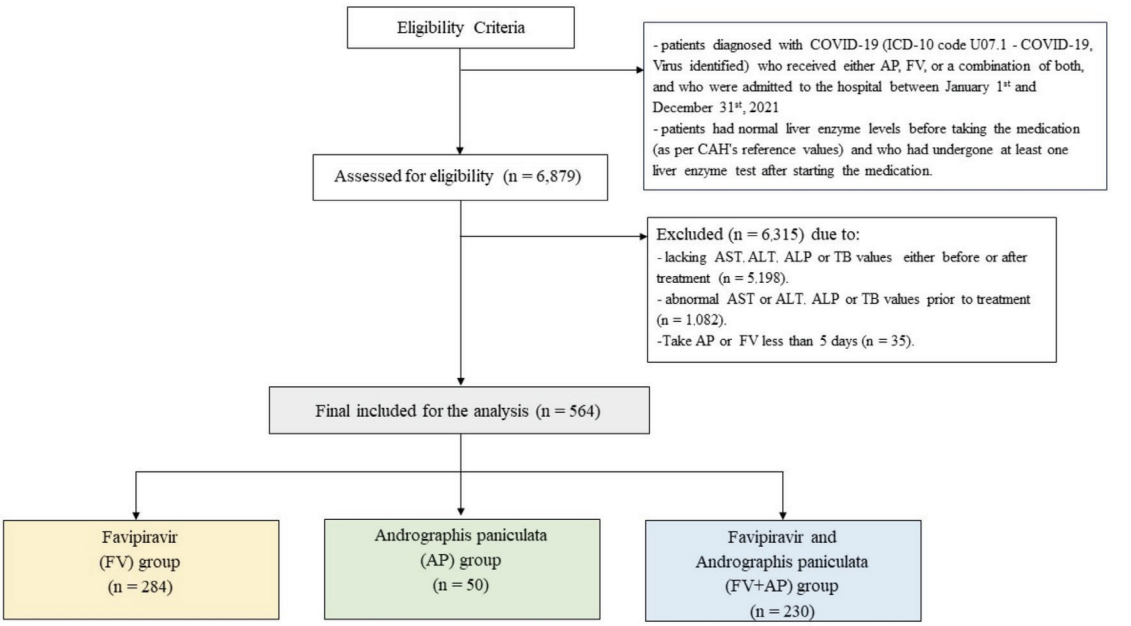

Figure 1 Study flow: Safety assessment of FV, AP AND FV+AP on liver function in COVID-19 patients

Results

Of the total eligible patients with COVID-19, only a subset of cases was considered for the analysis. Among these, 284 patients (50.35%), 50 patients (8.87%), and 230 patients (40.78%) received FV, AP, and FV+AP treatments, respectively. The basic characteristics of the patients, such as gender, age, body mass index, underlying diseases, smoking history, alcohol consumption history, history of

COVID-19 vaccination, co-medication and pre-treatment liver enzyme levels, are presented in Table 2.

Among the patients comprising the three groups, significant differences were identified with respect to three specific characteristics, namely mean age, comorbidities, and history of COVID-19 vaccination before treatment. Notably, the group of patients treated with AP had a relatively lower mean age of 33.36 ± 9.29 years, which was younger than the groups receiving FV and the combined FV+AP treatment, with mean ages of 44.81 ± 16.22 and 44.39 ± 15.65 years, respectively. Moreover, the percentage of patients with underlying diseases and a history of COVID-19 vaccination was highest in the FV group, followed by the FV+AP group, and then the AP group.

Differences in the administration of various medications for addressing the underlying diseases, symptoms of COVID-19, and its associated complications were also observed across the three distinct patient groups. The data demonstrates that the AP group exhibited the lowest utilization of medications for COVID-19 treatment, whereas the FV and FV+AP groups showed similar proportions in medication usage. Specifically, every patient within the FV group received medication for symptomatic relief, followed by 93.91% of patients in the FV+AP group, and 57.32% in the FV group. Regarding the management of COVID-19 complications, 60.92% of patients in the FV group, and 68.70% in the FV+AP group received medications, while merely 24.0% of patients in the AP group were administered such treatments.

For liver biochemistry, significant differences among the three groups were found only for ALP. The average ALP level in patients receiving AP was notably lower at 67.44 ± 12.80, followed by 70.93 ± 18.14 and 71.73 ± 16.12 in the FV+AP and FV groups, respectively

In terms of treatment, FV dosage was 63.37 ± 24.38 tablets (equivalent to 12.68 ± 4.88 grams), AP dosage was 60.80 ± 5.66 capsules (equivalent to 24.32 ± 2.26 grams), and for FV+AP, the dosage was 66.09 ± 20.45 tablets (equivalent to 13.22 ± 4.09 grams) and 60.80 ± 10.47 capsules (equivalent to 24.32 ± 4.19 grams), respectively.

The average follow-up duration after the last treatment for the three groups was found to be 5.41 ± 2.70, 5.36 ± 0.83, and 6.47 ± 3.26 days, respectively. There were no statistically significant differences among these durations (as depicted in Table 2).

Table 2 Patient characteristics

Characteristics	FV group (n = 284)	AP group (n = 50)	FV+AP group (n = 230)	*p*-value
Gender, n (%)				
Male	159 (55.99)	33 (66.00)	136 (59.13)	0.393
Female	125 (44.01)	17 (34.00)	94 (40.87)	
Age, Mean ± S.D. (years)	44.81 ± 16.22	33.36 ± 9.29	44.39 ±15.65	< 0.001*
Body Mass Index (BMI),				
Mean ± S.D. (kg/m^2)	24.51 ± 4.82	24.12 ± 5.40	25.22 ± 5.36	0.359
Underlying diseases, n (%)				
No	132 (46.48)	42 (84.00)	124 (53.91)	< 0.001*
Yes	152 (53.52)	8 (16.00)	106 (46.09)	
Hypertension	65 (22.89)	0 (0.00)	42 (18.26)	
Dyslipidemia	24 (8.45)	1 (2.00)	24 (10.43)	
Diabetes	15 (5.28)	0 (0.00)	13 (5.65)	
Chronic Kidney diseases	7 (2.46)	0 (0.00)	4 (1.74)	
Hepatitis	6 (2.11)	0 (0.00)	1 (0.43)	
Cerebrovascular diseases	4 (1.41)	0 (0.00)	1 (0.43)	
Tuberculosis	4 (1.41)	0 (0.00)	2 (0.87)	
Cancer	1 (0.35)	0 (0.00)	2 (0.87)	
Obesity	88 (30.99)	5 (10.00)	72 (31.30)	
Immunosuppression	4 (1.41)	0 (0.00)	1 (0.43)	
HIV-positive	5 (1.76)	0 (0.00)	0 (0.00)	
Gallstone	2 (0.70)	0 (0.00)	2 (0.87)	
Smoking history, n (%)				
No	201 (96.63)	26 (89.66)	152 (97.44)	0.124
Yes	7 (3.37)	3 (10.34)	4 (2.56)	
Alcohol drinking history, n (%)				
No	200 (94.34)	26 (89.66)	161 (96.99)	0.148
Yes	12 (5.66)	3 (10.34)	5 (3.01)	
COVID-19 vaccination, n (%)				
No	138 (48.59)	39 (78.00)	153 (66.52)	< 0.001*
Yes	146 (51.41)	11 (22.00)	77 (33.48)	
Comedication, n (%)				
For treatment of underlying diseases	75 (26.41)	3 (6.00)	54 (23.48)	0.003*
For treatment of COVID-19 symptoms	248 (57.32)	50 (100.00)	216 (93.91)	0.001*
For treatment of COVID-19 complications	173 (60.92)	12 (24.00)	158 (68.70)	< 0.001*
Liver enzyme (Before treatment)				
AST, Mean ± S.D. (U/L)	29.62 ± 7.01	27.98 ± 7.04	30.77 ± 7.18	0.928
ALT, Mean ± S.D. (U/L)	21.63 ± 9.53	22.70 ± 10.75	22.62 ± 10.03	0.465
ALP, Mean ± S.D. (U/L)	71.73 ± 16.12	67.44 ± 12.80	70.93 ± 18.14	0.007*
Bilirubin, Mean ± S.D. (mg/dL)	0.42 ± 0.19	0.39 ± 0.18	0.41 ± 0.19	0.766
Dose of medicine				
FV, (No. of tablets)	63.37 ± 24.38 (12.68 ± 4.88)	-	66.09 ± 20.45 (13.22 ± 4.09)	
AP, (No. of capsules)	-	60.80 ± 5.66 (24.32 ± 2.26)	60.80 ± 10.47 (24.32 ± 4.19)	
Duration of Laboratory Test Follow-up,				
Mean ± S.D. (Days)	5.41 ± 2.70	5.36 ± 0.83	6.47 ± 3.26	< 0.001*

Note: *Statistical significance (*p*-value < 0.05)

AST Monitoring

In terms of the follow-up on AST levels after treatment, it was noted that patients in all three groups exhibited median (interquartile range (IQR)) post-treatment levels of 28 (23-33), 24 (22-31), and 26 (22-35) U/L, respectively. Significant differences among the three groups were noted in the average AST changes before and after treatment, measuring 1.69 ± 15.51, -0.80 ± 7.96, and 0.01 ± 15.82 U/L in the FV, AP, and FV+AP groups, respectively. The number of individuals with elevated AST levels that exceeded the ULN as indicated in Table 3, was 33 (11.62%), 3 (6.00%), and 25 (10.87%) in the FV, AP, and FV+AP groups, respectively. No patients in the AP group had AST levels elevated by more than two times the ULN. However, there were 6 cases (2.11%) and 4 cases (1.74%) in the FV and FV+AP groups, respectively, where the AST value was approximately 2-3 times higher than the ULN. Additionally, 2 cases (0.87%) in the FV+AP group exhibited AST levels exceeding three times the ULN.

ALT Monitoring

Upon monitoring the post-treatment ALT values, it was observed that the median ALT (IQR) levels for the three groups were 24 (17-39), 22 (16-30), and 24 (16-39) U/L, respectively. The average ALT differences (before and after treatment) were notably significant at 10.87 ± 26.04, 3.08 ± 11.54, and 11.07 ± 26.90 U/L in the FV, AP, and FV+AP groups, respectively. No significant differences were noted among the groups in terms of patients with elevated ALT levels beyond the ULN at 57 (20.07%), 6 (12.00%), and 40 individuals (17.39%). Remarkably, no patients in the AP group exhibited ALT levels exceeding two times the ULN. Meanwhile, within the patient cohort, 10 cases (3.52%) and 13 cases (5.65%) in the FV and FV+AP groups, respectively, demonstrated ALT values that were approximately 2-3 times the ULN. Additionally, the occurrence of individuals with ALT values exceeding three times the ULN was observed in 3 cases (1.06%) in the FV group, and 5 cases (2.17%) in the FV+AP group.

ALP Monitoring

During the monitoring of post-treatment ALP values, it was observed that the average ALP levels for the three groups were 73.46 ± 18.77, 66.94 ± 13.34, and 72.73 ± 21.10 U/L, respectively, revealing statistically significant differences among the groups. The average ALP differences (before and after treatment) were 10.72 ± 12.95, -0.50 ± 6.67, and 1.80 ± 13.76 U/L, respectively, demonstrating notable variations among the three groups. Notably, no patients in the AP group exhibited an increase in ALP after treatment. On the other hand, in both the FV and FV+AP groups, the number

of individuals with values exceeding the ULN was 8 cases (2.82%) and 8 cases (3.48%). Furthermore, none of the patients in any of the groups demonstrated an increase in ALP levels exceeding two times the baseline value.

TB Monitoring

Upon monitoring the post-treatment TB values, it was observed that the average TB levels for the three groups were 0.46 ± 0.23, 0.54 ± 0.22, and 0.47 ± 0.22 U/L, respectively, revealing no significant differences among the groups. The average TB differences (before and after treatment) were 0.04 ± 0.22, 0.14 ± 0.21, and 0.05 ± 0.20 U/L, respectively, showcasing prominent variations across the three groups. Notably, no patients in the AP group exhibited elevated TB levels after treatment, while, in the remaining groups, elevated TB levels were observed in 4 individuals (1.41%) in the FV group, and 1 individual (0.43 in the FV+AP group. However, no one had elevated TB exceeding 2 times ULN. The after-treatment data on liver enzymes in each group are presented in Table 3.

Regarding drug-induced liver injury (DILI)[12], there were two cases found; one each in the FV and FV+AP groups. For the first case in the FV group, the patient received 90 tablets of FV over 10 days. Prior to treatment, the ALT value was 22 U/L, and after treatment, it increased to 296 U/L, which was an increase of 5.92 times from ULN within a 25-day period from the pre-treatment blood test. The other case occurred in the FV+AP group, where the patient received 60 capsules of AP in 5 days, and 90 tablets of FV in a period of 10 days. Prior to treatment, the ALT value was 18 U/L, and after treatment it rose to 216 U/L, which was 6.17 times the ULN within a 7-day period from the pre-treatment blood test. Further details of the patients' treatment are provided in Table 4.

Factors Affecting elevated Liver Enzyme Levels

Using binary logistic regression analysis, factors associated with heightened liver enzymes in COVID-19 patients receiving FV and AP were examined; nine potential factors that might influence liver enzyme elevation were considered. These factors encompassed gender, age, body mass index, underlying diseases, hepatitis, concomitant medications metabolized in the liver, history of alcohol consumption, history of smoking, and COVID-19 vaccination status.

The study revealed that across all three groups, no discernible factors were found to increase the probability of abnormal liver enzymes. However, the AP group did not meet the requirements for statistical significance

Table 3 Follow-up of liver enzyme values among groups

Follow-up of Liver Enzyme	FV group (n = 284)	AP group (n = 50)	FV+AP group (n = 230)	*p*-value
AST value after treatment				
AST, median (IQR) U/L	28 (23-33)	24 (22-31)	26 (22-35)	0.082
AST difference (before & after treatment), Mean ± S.D. (U/L)	1.69 ± 15.51	-0.80 ± 7.96	0.01 ± 15.82	< 0.001*
Patients with elevated AST (n,%)	33 (11.62)	3 (6.00)	25 (10.87)	0.572
Patients with elevated AST ~ 2-3 times from upper limit (n,%)	6 (2.11)	0 (0.00)	4 (1.74)	
Patients with elevated AST more than 3 times from upper limit (n, %)	0 (.00)	0 (0.00)	2 (0.87)	
ALT value after treatment				
ALT, median (IQR) U/L	24 (17-39)	22 (16-30)	24 (16-39)	0.419
ALT difference (before & after treatment), Mean ± S.D. (U/L)	10.87 ± 26.04	3.08 ± 11.54	11.07 ± 26.90	< 0.001*
Patients with elevated ALT (n, %)	57 (20.07)	6 (12.00)	40 (17.39)	0.376
Patients with elevated ALT ~ 2-3 times from upper limit (n, %)	10 (3.52)	0 (0.00)	13 (5.65)	
Patients with elevated ALT more than 3 times from upper limit (n, %)	3 (1.06)	0 (0.00)	5 (2.17)	
ALP value after treatment				
ALP, Mean ± S.D. (U/L)	73.46 ± 18.77	66.94 ± 13.34	72.73 ± 21.10	0.001*
ALP difference (before & after treatment), Mean ± S.D. (U/L)	1.72 ± 12.95	-0.50 ± 6.67	1.80 ± 13.76	< 0.001*
Patients with elevated ALP (n, %)	8 (2.82)	0 (0.00)	8 (3.48)	
Patients with elevated ALP ~ 2-3 times from upper limit (n, %)	0 (0.00)	0 (0.00)	0 (0.00)	-
Patients with elevated ALP more than 3 times from upper limit (n, %)	0 (0.00)	0 (0.00)	0 (0.00)	-
Total Bilirubin value after treatment				
Bilirubin, Mean ± S.D. (mg/dL)	0.46 ± 0.23	0.54 ± 0.22	0.47 ± 0.22	0.431
Bilirubin difference (before & after treatment), Mean ± S.D. (mg/dL)	0.04 ± 0.22	0.14 ± 0.21	0.05 ± 0.20	0.461
Patients with elevated bilirubin (n, %)	4 (1.41)	0 (0.00)	1 (0.43)	
Patients with elevated bilirubin ~ 2-3 times from upper limit (n, %)	0 (0.00)	0 (0.00)	0 (0.00)	-
Patients with elevated bilirubin more than 3 times from upper limit (n, %)	0 (0.00)	0 (0.00)	0 (0.00)	-

Note: * Statistical significance (*p*-value < 0.05)

Table 4 Follow-up of patients after treatment eligible for drug-induced liver injury

Patients	Hospital length of stay (days)	Underlying diseases	Medicine	Laboratory check	AST	ALT	ALP	Bilirubin
Male aged	27	Hypertension,	FV 90 Tab for	27 Oct 21	36	22	86	0.6
36 y/o		Obesity	10 days	30 Oct 21	33	22	103	0.4
				4 Nov 21	59	90	77	0.7
				20 Nov 21	107	296	82	1.6
– Other medications received during treatment include; calpolystyrene powder 5 gm, insulin mixed 30/70 iu/ml (winsulin-30/70), humulin n (nph) 100 iu/ml, humulin r (ri insulin) 100 iu/ml, dexamethasone inj 5 mg/ml, glucose 50 ml, hyoscine (buscopan) 20 mg/ml, methylprednisolone 1gm, metoclopramide hcl inj 10 mg/2 ml 10 mg/2 ml, omeprazole (losec) 20- 40 mg, potassium chloride 10%, sodium chloride (nss/2) 0.45%, budesonide mdi 200 mcg/dose, metformin (glucophage) 500 mg, ceftriaxone (cef-3) 1 g, albendazole 200 mg, d-5-s/2 (5%d/n/2) inj, glyceryl guiacolate (gg) 100 mg, nss 50 ml inj in bag 100 ml , paracetamol 500 mg, prednisolone 5 mg, simeticone (air-x) 80 mg, sodium chloride iv (nss) inj , sterile water for inj, phyllanthus cough syrup								
– Diagnosis: hyponatremia, hypokalemia, acidosis, toxic liver disease with acute hepatitis, antiviral drugs adverse effect, asphyxia								
Female aged	25	No	1) AP 60 cap for 5 days	18 Aug 21	28	18	79	0.4
66 y/o			2) FV 90 tab for 10 days	25 Aug 21	120	216	125	0.7
– Other medications received during treatment include; dexamethasone inj 5 mg/ml, hydrocortisone (solu cortef) 100 mg/2 ml, levofloxacin (cravit) 750 mg/150 ml, amlodipine 5 mg, budesonide mdi 200 mcg/dose, ceftriaxone (cef-3) 1 g, cetirizine (zyrtec) 10 mg, ephedrine nasal drop 1%, glyceryl guiacolate (gg) 100 mg, ipratropium+fenoterol (aerobidol mdi), lorazepam (ativan) 1 mg, paracetamol 500 mg, prednisolone 5 mg, sodium chloride iv (nss) inj 0.9%, sterile water for inj, urea cream 10%								
– Diagnosis: Septicaemia due to other specified staphylococcus, insomnia, asphyxia, xerosis cutis (dry skin)								

due to the limited number of patients in this cohort, and a similar frequency of cases with abnormal liver enzymes was observed post-treatment (data not presented).

Discussion

The results of this study regarding liver toxicity do not support the statement "It is not recommended to concomitantly use AP (in asymptomatic COVID-19) with antiviral due to potential serious side effect" in the National CPG for the cases of coronavirus disease 2019, version 4 August 2021 to version 1 March 2022. This is based on the findings that the patients in the FV groups exhibited the highest rates of elevated AST, ALT, and TB, at 11.62%, 20.07%, and 1.41%, respectively, while those in the FV+AP group demonstrated slightly lower abnormal levels, at 10.87%, 17.39%, and 043%, respectively. Conversely, FV+AP group presented the highest proportion of patients with elevated ALP levels, at 3.48%, surpassing

those in the FV group (2.82%); however, such elevated ALP levels were not observed in the AP group.

However, the utilization of FV alone or in combination with AP led to a substantial increase in ALT levels and was associated with DILI in 2 cases. It cannot be definitively concluded that FV alone or in combination with AP was the direct cause of the observed ALT elevation and DILI. The first patient with the elevated levels had underlying conditions like diabetes and obesity, and had uncontrolled blood sugar at the time of admission. The patient was administered various medications, such as levofloxacin, budesonide, lorazepam, amlodipine, paracetamol and omeprazole, which have been associated with increase chances of liver abnormality[13-14]. The second patient, at 66 years old, faced age-related complications despite having no underlying diseases. Furthermore, both patients received FV for a duration of 10 days, aligning with existing research indicating that FV administration is linked to incidences of liver function abnormalities in COVID-19 patients, ranging from 6.8% to 44%. High doses of FV are associated with higher likelihood of liver function abnormality[15]. Therefore, our findings imply that the observed alterations in liver enzymes may have been influenced by the presence of comorbidities and concurrent medication, particularly those metabolized in the liver, rather than being solely attributable to the administration of antiviral medications like FV and AP.

The cases of hepatitis identified in our study align with those reported in a recent clinical trial (APFaVi trial) involving administration of AP extract (AP) plus favipiravir (FV) or placebo plus favipiravir in two groups of non-severe COVID-19 patients.[16] However, in the APFaVi trial, AP was administered at the dose of 180 mg andrographolide per day for 5 days. In that trial, one case (1.75%) in AP+FV group exhibited elevated ALT levels exceeding three times the upper limit of normal (ULN) on day 5 of treatment, classified as presenting mild hepatitis. Moreover, mild hepatitis was reported in 14 patients (24.56%) in both group. But in the combined therapy of AP and FV with in those receiving FV and a placebo had hepatitis increased on day 14 of the study. All individuals with hepatitis were able to recover by day 28. In the FV with a placebo group, three cases displayed an increase in ALT levels ranging from 3 to 5 times the ULN (moderate hepatitis) on day 14. However, the baseline of liver enzymes, follow-up, comedication was not presented and other factors that might cause additional hepatitis after 14 days of treatment. If sufficient data were available, further studies could potentially identify the causes in the future. Our study analyzed factors influencing abnormal liver enzyme elevation, but found no significant factors that

would cause an increase in abnormal liver enzymes.

Interestingly, our study found that the utilization of AP with andrographolide 144 mg/day alone in young patients without underlying diseases did not lead to significant elevation in liver enzymes. This finding is consistent with previous reports by Wanaratna[17] and Benjaponpitak[18]. Furthermore, the data may suggest a pattern of physicians lacking confidence in prescribing AP, as it appears that, when AP was administered, patients simultaneously received other medications for symptomatic relief despite the potential benefits of AP in alleviating symptoms associated with COVID-19[19]. Evidence synthesis should be conducted to support the use of AP.

One limitation of our study is its retrospective design, which made it difficult to exclude confounding factors influencing liver function. The observed liver enzyme abnormality may be attributed to other factors beyond FV or AP alone. Moreover, the relatively small number of patients, especially in the AP group, constrained our ability to establish definitive associations between these factors and the elevation of liver enzymes.

Conclusion

The study findings indicate that 0.35% of patients in the FV group and 0.434% in the FV+AP group exhibited AST and ALT levels exceeding three times ULN, making them prone to DILI. Consequently, careful monitoring of liver function is essential for the use of both FV and FV+AP, especially in patients of advanced age, those with underlying diseases, and those taking medications metabolized in the liver. Additionally, the administration of AP at a dosage of andrographolide 144 mg/day demonstrated a favorable safety profile for liver enzymes, suggesting its potential suitability for prescribing for young patients without underlying diseases. This data also supports the use of andrographolide in common cold or influenza, as the dose used in these two indications is one-third of that used in COVID-19. Future research efforts should prioritize the synthesis of comprehensive evidence to strengthen physician confidence in prescribing andrographolide.

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