



# An experimental investigation evaluating the protective effect of a hydroalcoholic extract of the aerial portion of *Alenda (Ephedra fragilis* Desf.) against hepatotoxicity caused by mercaptopurine in rats

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## ABSTRACT

Chemical hepatotoxicity can lead to various liver illnesses, including hepatitis and cirrhosis. Mercaptopurine (MP) is a potent anti-tumor medication, but its use is restricted due to its potential adverse effects, such as myelotoxicity. Plants naturally contain hepatoprotective phytoconstituents that protect the liver from hepatotoxicity and promote liver regeneration. The purpose of the study is to investigate the potential protective impact of hydroalcoholic aerial component extract from *Ephedra fragilis Alenda (E. fragilis)* against MP-induced liver damage in rats. Rats were randomly assigned to seven groups. Silymarin at 50-mg.kg<sup>-1</sup> b.w., MP at 40-mg.kg<sup>-1</sup> b.w., *E. fragilis* extract 30-mg.kg<sup>-1</sup> b.w., *E. fragilis* extract 30 mg.kg<sup>-1</sup> and MP 40 mg.kg<sup>-1</sup>, Silymarin 50 mg.kg<sup>-1</sup> and MP 40 mg.kg<sup>-1</sup>, *E. fragilis* extract (15 mg.kg<sup>-1</sup>), silymarin dose of 25 mg.kg<sup>-1</sup>, and MP 40 mg.kg<sup>-1</sup> were given to the control group. On days 0, 10, and 21, blood samples were taken. The study used serum biochemical markers such as albumin, bilirubin, liver enzymes, and total protein to measure liver damage and histopathological alterations. In rats treated with MP, the administration of *E. fragilis* extract decreased bilirubin and alkaline phosphatase levels. Histopathological analysis of livers revealed that *E. fragilis* extract decreased necrosis, fatty degeneration, and anadation in MP-treated rats. These findings indicate that *E. fragilis* extract potentially protects against liver injury in rats caused by MP. It has a possible protective effect against drug-induced cholestasis. These findings show that *Alenda* extract might be an effective hepatoprotective agent for improving hepatocellular damage.

## INTRODUCTION

Mercaptopurine (MP) is a purine analog that prevents the creation of RNA and proteins and is an antagonist to natural purines necessary for DNA replication [1]. MP treats leukemia, inflammation, and cancer and inhibits the immune system. Its usage is limited, though, because of severe adverse effects such as gastrointestinal distress, bone marrow suppression, and liver damage [2]. Lymphocytes absorb the prodrug after being metabolized in the gastrointestinal tract system and liver. The

purine salvage enzyme breaks down MP to produce thioguanine nucleotides and thioinosine monophosphate [3]. The amount of purines available for DNA synthesis is further decreased by methylthioinosine monophosphate, a potent purine de novo synthesis inhibitor. When synthesized thiopurine nucleotides replace native purines, cells experience purine deprivation, which stops the production of DNA, RNA, and proteins and reduces cell proliferation and cytotoxicity [4,5].

The metabolites thioguanine can harm myeloid, liver, and renal tissue without benefitting life [4,5]. Both acute and long-term MP consumption can cause hepatotoxicity. During the first several months of treatment, jaundice, tiredness, and increased enzymes are common symptoms of acute liver damage [4]. Persistent therapy may lead to the development of portal hypertension and nodular regenerative hyperplasia, which

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would ultimately cause liver failure. When thiopurines, such as MP, suppress the immune system in males with inflammatory bowel disease, an uncommon consequence is hepatosplenic T-cell lymphoma [3].

The adverse effects of synthetic medications are drawing attention to the therapeutic evaluation of medicinal plants using a systematic research approach. Since the conventional treatment of liver issues is associated with a number of detrimental side effects, botanical medications are commonly used [6].

*Ephedra fragilis* (*E. fragilis*) is a member of the Ephedraceae botanical family. This family's plants are well known for their many ephedrine alkaloids, the most prominent of which are ephedrine and pseudoephedrine [6,7]. The primary phytochemical components of *Ephedra* plants that are thought to be responsible for these pharmacological characteristics are flavonoids, alkaloids, phenolic acids, and other chemicals. The *Ephedra* plant contains more than 80% alkaloids, including ephedrine and pseudoephedrine. A study on one *Ephedra* species discovered hydroalcoholic extract had a more significant impact than aqueous extract and had higher antioxidant, anti-inflammatory, and antiviral properties [7,8]. *Ephedra fragilis* and *Ephedra Altea*, used interchangeably for the same common name Alenda in Jordan, are very stimulating plant that has been used for a long time in Jordan under the name Alenda or pine joints [9]. It is also well known and respected in the herbalist traditions of many other nations. It is widely utilized and well-studied in the Tefillah area in southern Jordan, where its anticancer properties have been established in multiple studies [8–10].

The liver is an essential organ that manages chemical metabolism, secretion, storage, and detoxification [11]. Liver cirrhosis is the leading cause of death in hepatic disorders, which represents a severe threat to public health. Even with the advances in contemporary medicine, no entirely effective drugs can protect the entire organ or improve liver function. Plant-based phytochemicals are crucial to the search for safer and more productive pharmaceutical substitutes. Viral infections, malignancies, alcoholism, drug overdoses, and nonalcoholic fatty liver disease are examples of hepatic illnesses. Treatment-induced liver impairment can result in treatment withdrawal, and liver transplantation is necessary for the majority of end-stage liver disease situations. Hepatoprotective herbs, such as milk thistle, turmeric, and N-acetylcysteine, can potentially prevent or treat liver damage [12]. The hepatoprotective ability of *E. fragilis*, Alenda, against MP consumption has not been the subject of any scientific investigation, previously according to published research. Few investigations have established the detrimental effects of *E. fragilis* and the efficacy of several species of *Ephedra* as hepatoprotective activity.

This research aims to assess the hepatoprotective and antioxidant properties of the aerial parts of the *E. fragilis* plant. There is a link between oxidative stress and inflammation; free radicals can promote inflammation [8]. Thus, this work assessed the hepatoprotective qualities of *E. fragilis* aerial portion utilizing MP-intoxicated rats as an experimental paradigm.

## EXPERIMENT

### Chemicals

The Sigma-Aldrich Company provided the analytical reagent grade chemicals and used reagents.

### Plant material

Mutah University authenticated the *E. fragilis* plant, which was harvested on March 1st, 2022, and 2023, from Tafillah, Jordan, authenticated by Dr. Saleh Al-Quran (Department of Biology, Mutah University, Karak, Jordan). A drug development lab received the voucher specimen and assigned specimen number ER008 for deposit. The aerial portion was dried, ground for a short while, and then stored in the dark at room temperature. The mixture was filtered using filter paper 48 hours after 33% ethanol was added. The resultant extracts were then concentrated at  $40^{\circ}\text{C} \pm 5^{\circ}\text{C}$  under reduced pressure and then lyophilized for 48 hours [13].

### Preliminary phytochemical screening

Using conventional techniques, phytochemical screening assays were carried out to determine if phytoconstituents were present in *E. fragilis* extracts.

The study focuses on detecting tannins, saponins, alkaloids, and flavonoids in plant extracts. Distilled water and ferric chloride are added to a plant extract to produce blue and green/black colors, which are used to evaluate the tannin content. A layer of foam detects saponins. Alkaloids are calculated by adding 2% hydrochloric acid to the residue in a water bath at  $100^{\circ}\text{C}$ . After filtering, the mixture is split into two equal parts. Sodium hydroxide is added to a solution to detect flavonoids and flavones, causing the solution to become yellow. Following treatment with 5N hydrochloric acid, the solution becomes orange for flavones and colorless for flavonoids [13].

### Determination of phenolic

The total phenol content of the hydroalcoholic extract of *E. fragilis* was determined using the Folin–Ciocalteu technique. About 200  $\mu\text{l}$  of the sample solution and 250  $\mu\text{l}$  of 1.0-N Folin's reagent were combined. After 5 minutes, approximately 1,300  $\mu\text{l}$  of 20% sodium  $\text{Na}_2\text{CO}_3$  was added, and the mixture was vigorously agitated. Using UV/visible (double beam Perkin Elmer spectrophotometer), the absorbance was measured at  $760 \pm 2$  nm following 45 minutes of incubation at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . A calibration curve was made using gallic acid standards. Gallic acid equivalents (GEs) expressed the phenolic content [13,14].

### Determination of flavonoids

Aluminum chloride and a hydroalcoholic sample solution determined the total flavonoid content. After 35 minutes of incubation at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , the samples' absorbance at  $500 \pm 5$  nm was determined. Standard quantities of a quercetin methanolic solution were used to generate a calibration curve, and the total flavonoid content was expressed using quercetin equivalents or QE [13,14].

### Free radical scavenging activity/2,2-diphenyl-1-picrylhydrazyl

The study used 2,2-diphenyl-1-picrylhydrazyl (DPPH) to measure free radical scavenging activity in extract and reference

samples. The samples were treated with DPPH ethanolic solution and then subjected to absorbance measurements at  $520 \pm 2$  nm. Ascorbic acid is the standard, and an ethanolic DPPH solution was used as a negative control [13,14].

### Animals

In this study, laboratory-bred Wistar rats (female) weighing 150–180 g were procured from the Laboratory Animal Research Center of the Applied Science University located/in Amman, Jordan. For the duration of the experiment, they were kept in a facility with a constant temperature of  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and regular cycles of light and dark (12/12 h). The Applied Science University Faculty of Pharmacy's ethics committee approved all experimental protocols and techniques intending to lessen the suffering of the animals (Ethics approval references number: 2023-PHA-57). These rules adhered to globally acknowledged norms that govern the care and use of animals in experiments [14,15].

### In vivo study

Rats were divided into seven groups. Each group comprised six rats, and each group received MP, Silymarin, and *E. fragilis* orally for 21 days following Table 1 [14–18]. The positive control group was the silymarin group, while the

negative control group was the hydroalcoholic water. Doses were selected according to reported safe values for *E. fragilis* extract, while therapeutic doses were previously used for MP and Silymarin [14–19].

As illustrated in Table 1, animal groups were treated as follows—C: normal control (rats treated with 1 ml/day of hydroalcoholic water); MP: mp (40 mg/kg /day); S: silymarin (50 mg/kg); E: *E. fragilis*; extract (30 mg/Kg); E/MP: *E. fragilis*; extract (30 mg/Kg) and MP (40 mg/kg); S/MP: silymarin (50 mg/kg) and MP (40 mg/kg); E/S/MP: *E. fragilis*; extract (15 mg/Kg); silymarin (25 mg/kg); and MP (20 mg/kg). The protocol adhered to several published protocols evaluating hepatoprotective activity for plant extracts using silymarin as standard material for its hepatoprotective activity at a dose of 50-mg/kg body weight [15]. Ephedra dose was selected according to previously reported hepatoprotective dose against deltamethrin hepatic injury with a dose of about 30-mg/kg body weight in conjunction with the control group administered the same dose for more pronounced safety evaluation [10,15].

### Assessment of hepatic injury (biochemical markers)

Conventional biomarkers for liver injury fall into two categories: those that indicate a disturbance in the liver's homeostasis or normal function and those that offer distinct indications of cellular and tissue damage [11]. The liver processes bile acids and other endogenous compounds, synthesizes proteins, and excretes metabolic waste products including urea and bilirubin. Conventional indicators of compromised liver function include changes in plasma bile acids, plasma total bilirubin (TB), and plasma total plasma protein brought on by medications or illnesses [1–4]. These indicators may be evaluated because injured or dying cells frequently release them into the bloodstream [6–10,12–19]. This group of enzymes includes gamma-glutamyl transferase, glutamate dehydrogenase, and aspartate aminotransferase (SGPT). Other readily available alternatives include triglycerides, albumin (ALB), and total protein (TP). Accordingly, these indicators are used in this study.

**Table 1.** Experiment design. Seven groups of six rats each were set up in a random manner.

Group ID	Treatment
C	Control received only hydroalcoholic water and food.
MP	MP at 40-mg/kg body weight.
S	Silymarin at 50-mg/kg body weight.
E	<i>E. fragilis</i> Aerial part extract 30-mg/kg body weight.
E/MP	<i>E. fragilis</i> Aerial part extract 30-mg/kg and MP 40 mg/kg.
S/MP	Silymarin 50-mg/kg and MP 40 mg/kg.
E/S/MP	<i>E. fragilis</i> Aerial part extract 15 mg/kg, silymarin 25 mg/kg, and MP 40 mg/kg.

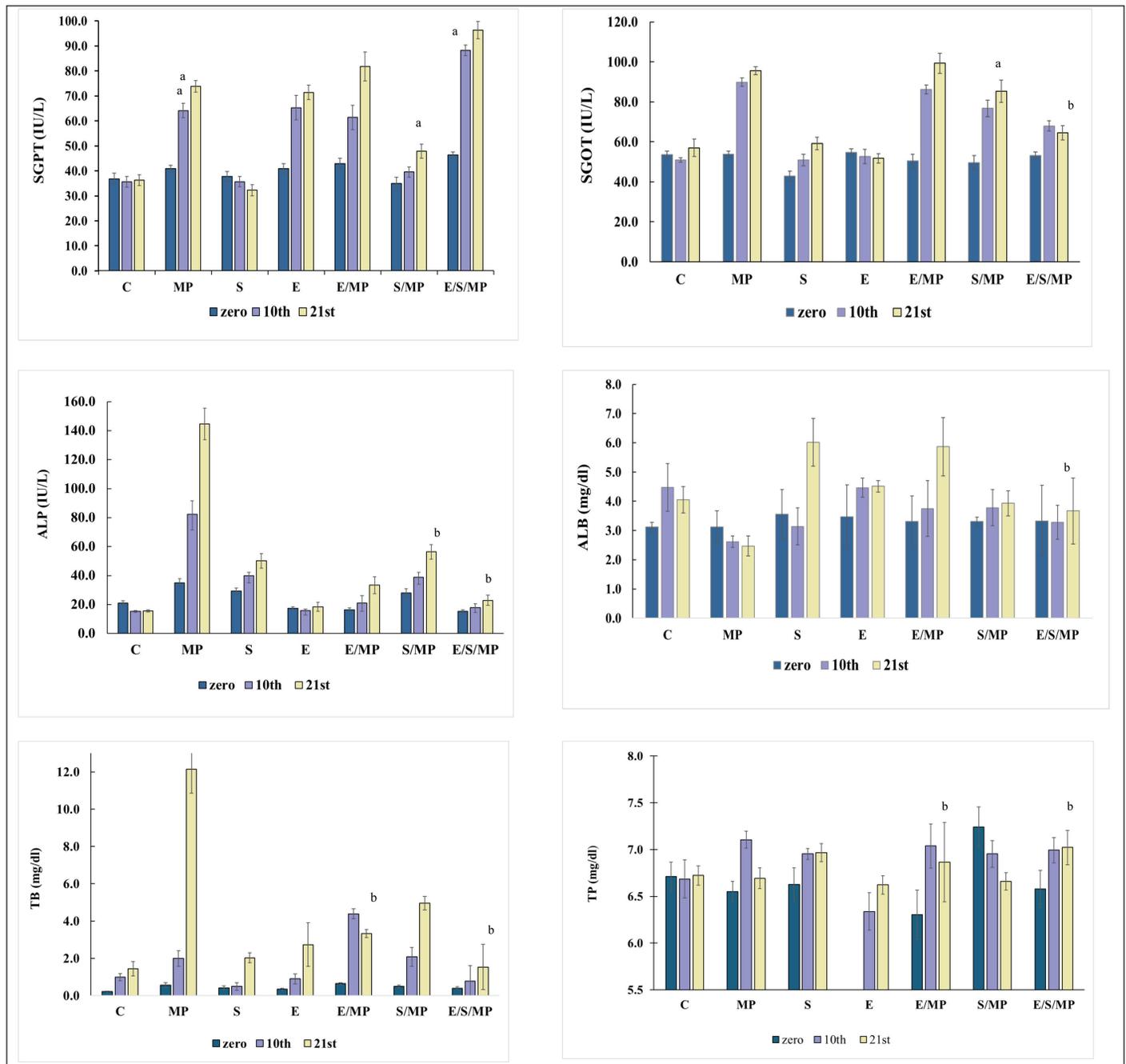
**Table 2.** Effect of oral administration of *E. fragilis* Aerial part hydroalcoholic extract at a dose of 30-mg/kg body weight on serum AST, ALT, ALP, TB, ALB, and TP serum levels in MP-induced hepatotoxicity in rats at day 21. Each column represents mean  $\pm$  SEM for 6 rats.

Group*	SGPT (IU/l)	SGOT (IU/l)	ALP (IU/l)	ALB (mg/l)	TB (mg/l)	TP (mg/l)
C	36.2 $\pm$ 2.0	57.0 $\pm$ 4.3	15.6 $\pm$ 0.8	4.0 $\pm$ 0.3	1.4 $\pm$ 0.2	6.7 $\pm$ 0.2
MP	73.8 $\pm$ 2.2 <sup>c</sup>	95.6 $\pm$ 1.9 <sup>c</sup>	144.6 $\pm$ 40.8 <sup>c</sup>	2.5 $\pm$ 0.2	12.1 $\pm$ 1.1	6.7 $\pm$ 0.2
S	32.2 $\pm$ 2.0	59.2 $\pm$ 3.1	50.1 $\pm$ 4.9	6.0 $\pm$ 0.7	2.0 $\pm$ 0.1	7.0 $\pm$ 0.1
E	71.4 $\pm$ 2.3 <sup>c,d</sup>	51.7 $\pm$ 2.2	18.5 $\pm$ 2.0	4.5 $\pm$ 0.5	2.7 $\pm$ 0.6	6.6 $\pm$ 0.4
E/MP	84.8 $\pm$ 6.0 <sup>c</sup>	99.3 $\pm$ 10.6 <sup>c</sup>	33.3 $\pm$ 6.2 <sup>d</sup>	5.9 $\pm$ 0.5	3.3 $\pm$ 1.1	6.9 $\pm$ 0.4
S/MP	47.9 $\pm$ 3.1	85.3 $\pm$ 5.5	56.3 $\pm$ 5.0	3.9 $\pm$ 0.3	5.0 $\pm$ 0.2	6.7 $\pm$ 0.1
E/S/MP	96.4 $\pm$ 6.9	64.5 $\pm$ 2.5	22.9 $\pm$ 1.6	3.7 $\pm$ 0.4	1.5 $\pm$ 0.4	7.0 $\pm$ 0.1

a:  $p < 0.05$ ; b:  $p < 0.01$ ; c:  $p < 0.001$  compared to control group, C and d: insignificant compared to MP-treated group; C: Control received only hydroalcoholic water and food; MP:MP at 40-mg/kg body weight; S: Silymarin at 50-mg/kg body weight; E: *E. fragilis* extract 30-mg/kg body weight; E/MP: *E. fragilis* extract 30 mg/kg and MP 40 mg/kg; S/MP: Silymarin 50 mg/kg and MP 40 mg/kg; E/S/MP: *E. fragilis* extract 15 mg/kg; silymarin 25 mg/kg, and MP 40 mg/kg.

Blood samples were drawn from the rats' tail veins during the trial's first, tenth, and twenty-first days. Chloral hydrate was used to conduct animal anesthesia. The TP (Arcomex, Jordan), ALB, TB, glutamic-oxaloacetic transaminase (SGOT), SGPT, and alkaline phosphatase (ALP) (Beacons Diagnostic Pvt Ltd, India) diagnostic kits

were all evaluated [1–4, 6–10,12–19]. According to Eidi *et al.* [19], liver tissue samples were obtained for histological examination, and the same pathologist assessed and assigned a rating. The data analysis investigator is blinded to the fact that the specimens or animals served as controls and treatments.



**Figure 1.** Effect of oral administration of *E. fragilis* Aerial part hydroalcoholic extract at a dose of 30-mg.kg<sup>-1</sup> body weight on serum AST, ALT, ALP, TB, ALB, and TP serum levels in MP-induced hepatotoxicity in rats at days: 0, 10, and 21, for six rats. (A) and d: insignificant compared to MP-treated group (B); \*: C: Control received only distilled water and food; MP:MP at 40-mg.kg<sup>-1</sup> body weight; S: Silymarin at 50-mg.kg<sup>-1</sup> body weight; E: *E. fragilis* extract 30-mg.kg<sup>-1</sup> body weight; E/MP: *E. fragilis* extract 30 mg.kg<sup>-1</sup> and MP 40 mg.kg<sup>-1</sup>; S/MP: Silymarin 50 mg.kg<sup>-1</sup> and MP 40 mg.kg<sup>-1</sup>; E/S/MP: *E. fragilis* extract 15 mg.kg<sup>-1</sup>; silymarin 25 mg.kg<sup>-1</sup>, and MP 40 mg.kg<sup>-1</sup>. The error bars represent the SEM of measurements in six samples ( $n = 6$ ). \* a: significant difference compared to the C group at  $p$  values < 0.05, b: insignificant difference compared to the C group at  $p$  values > 0.05.

### Histopathology

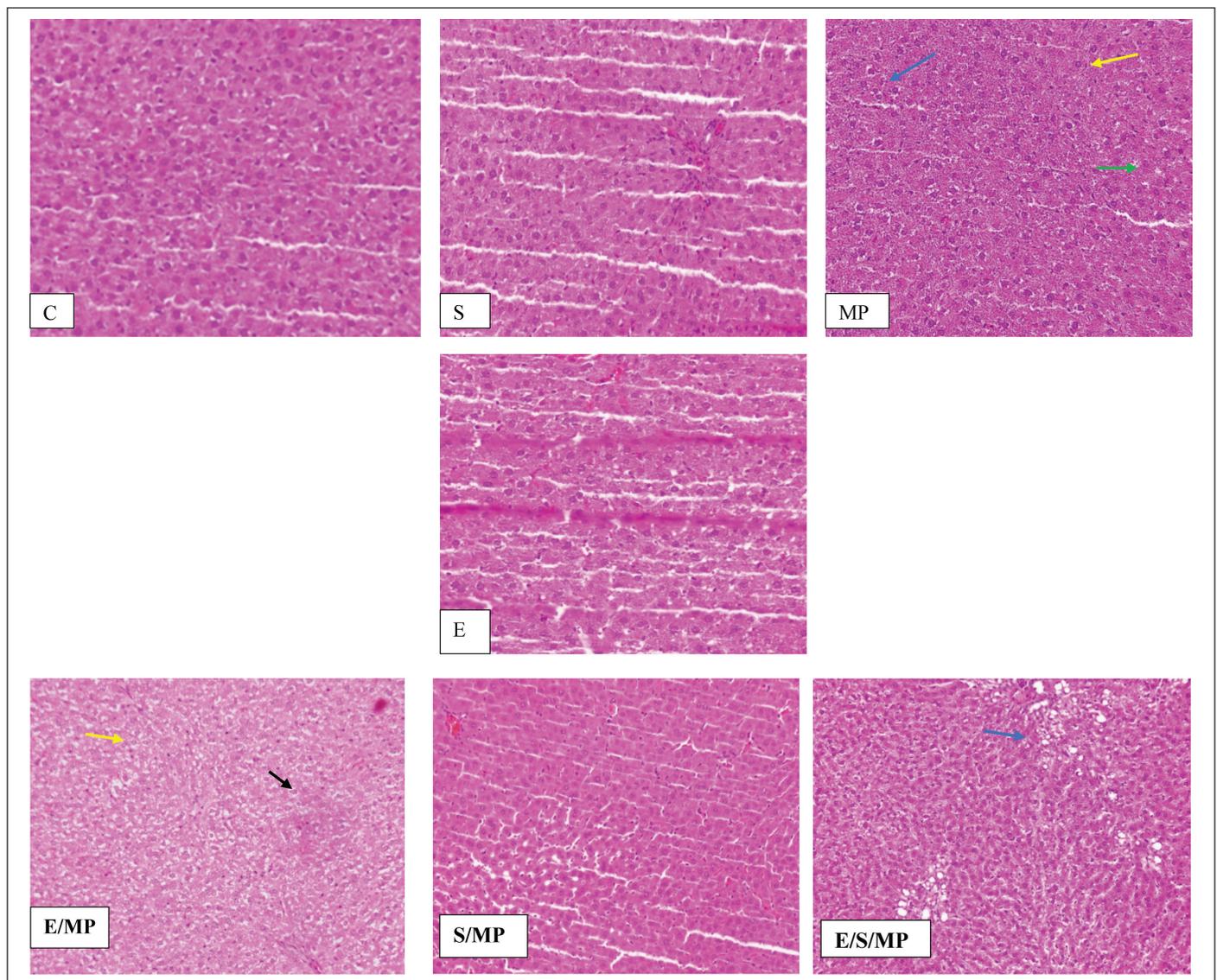
About 5- $\mu\text{m}$ -thick tissue slices and 10% formalin in saline solution were used for the histological analysis of the liver. After being dried in alcohol, the slices were wrapped in paraffin. A histopathologist will stain and evaluate the samples using hematoxylin and eosin (H&E). The liver slices were quantitatively graded to assess the acute hepatic injury [14].

A histopathologist used a light microscope to examine these slices after they had been stained with H&E, as previously mentioned in [15,19]. A quantitative scoring system was used to evaluate the histological characteristics of acute hepatic injury in the liver sections. Significant hepatocyte necrosis, lymphocyte and Kupffer cell infiltration, lipid alteration, and hyaline degeneration were all shown by the histological results.

Hyaline degeneration, lipid change, lymphocyte, and Kupffer cell infiltration, and considerable hepatocyte necrosis were among the histological findings. The French Metavir Cooperative Study Group [20] methodology for evaluating liver pathology was followed. A score of 0 indicates no discernible cell damage. Localized hepatocyte injury is seen in less than 25% of the tissue when the score is 1 [15,19].

### Statistical analysis

The SPSS22 program, Tukey's test, and the analysis of variance test were used to assess the results of the biochemical parameter analysis. Statistical significance was reached when the *p*-value, which shows a difference, was less than 0.05. Results expressed as average  $\pm$  standard error of the mean (Av



**Figure 2.** Hepatic tissue sections: (A) and d: insignificant compared to MP-treated group (B); \*: C: Control received only distilled water and food; MP:MP at 40-mg. $\text{kg}^{-1}$  body weight; S: Silymarin at 50-mg. $\text{kg}^{-1}$  body weight; E: E. fragilis extract 30-mg. $\text{kg}^{-1}$  body weight; E/MP: E. fragilis extract 30 mg. $\text{kg}^{-1}$  and MP 40 mg. $\text{kg}^{-1}$ ; S/MP: Silymarin 50 mg. $\text{kg}^{-1}$  and MP 40 mg. $\text{kg}^{-1}$ ; E/S/MP: E. fragilis extract 15 mg. $\text{kg}^{-1}$ ; silymarin 25 mg. $\text{kg}^{-1}$ , and MP 40 mg. $\text{kg}^{-1}$ . Blue arrow shows inflammation, yellow arrow shows edema, green arrow shows necrosis, and black arrow shows fatty degeneration.

± SEM). To assess for data normality and homogeneity, the Shapiro–Wilk and Levene tests were applied.

## RESULTS

### Phytochemical, total phenols, flavonoids, and DPPH analysis

Both Wagners and Dragendorff yield positive scores for alkaloids. Moreover, saponins and tannins produce positive results for the qualitative phytochemical investigation.

The *E. fragilis* ethanol:water (33%) extract had a total phenolic content of  $46.7 \pm 1.7$  mg GE per gram of dry extract (mean ± SEM). The flavonoid amount in 1 g of dry extract was  $76.6 \pm 1.3$  mg QE (mean ± SEM). Moreover, an  $IC_{50}$  of  $0.126$  mg.ml<sup>-1</sup> was obtained from the DPPH Free Radical Scavenging Assay. The extract yielded 3% w/w per gram of plant dry powder utilized.

### Serum biochemical parameters

As indicated in Table 2, the plasma biochemical parameters were assessed in each of the rats on days 0, 10, and 21 of the assessment. As demonstrated in Table 2 and Figure 1, rats treated with MP exhibited significantly higher levels of ALP on day ten and continued to increase substantially on day 21 ( $144.6$  IU.l<sup>-1</sup>) ( $p = 1 \times 10^{-7}$ ) of the study, as well as significantly higher levels of SGPT ( $73.8$  IU.l<sup>-1</sup>) and SGOT ( $95.6$  IU.l<sup>-1</sup>) on days 10 and 21 of evaluation ( $p = 5 \times 10^{-6}$ ;  $2 \times 10^{-5}$ ). ALB was reduced to  $2.5$  mg.l<sup>-1</sup>, although the difference was insignificant ( $p = 0.86$ ). On day 21 of the research, total bilirubin significantly increased to  $12.1$  mg.l<sup>-1</sup> ( $p = 9 \times 10^{-12}$ ).

However, compared to group C, the administration of *E. fragilis* significantly increased SGPT levels to  $71.4$  IU.l<sup>-1</sup> ( $p = 2 \times 10^{-5}$ ) and insignificantly different from the MP group ( $p = 1.0$ ) while maintaining other parameters as expected and no significant difference against the control group. Similarly, silymarin has no impact on all parameters, which shows its harmless effect on liver function. Combination E/MP increased SGPT  $84.8$  IU.l<sup>-1</sup> ( $p = 9 \times 10^{-13}$ ); SGOT  $99.3$  IU.l<sup>-1</sup> ( $p = 1 \times 10^{-8}$ ), low increment effect against ALP  $33.3$  IU.l<sup>-1</sup> not significant ( $p = 0.999$ ) compared to group C. TB did not increase significantly at  $3.3$  mg.l<sup>-1</sup> ( $p = 0.905$ ), TP  $6.9$  mg.l<sup>-1</sup>, and ALB  $5.9$  mg.l<sup>-1</sup> ( $p = 0.942$ ).

The silymarin combination shows a better effect on SGPT levels than E/MP, so it gets a value of  $47.9$  IU.l<sup>-1</sup>, while SGOT significantly increased to  $85.3$  IU.l<sup>-1</sup> ( $p = 0.005$ ), ALP  $56.3$  IU.l<sup>-1</sup> not significantly higher than C ( $p = 0.576$ ). Similarly, normal levels were obtained for ALB, TB, and TP. Furthermore, triple combination E/S/MP shows different behavior; SGPT significantly increased  $96.4$  IU.l<sup>-1</sup> ( $p = 9 \times 10^{-13}$ ), while there was no significant increase in all other parameters: SGOT  $64.5$  IU.l<sup>-1</sup>, TP  $7$  mg.l<sup>-1</sup>, ALP  $22.9$  IU.l<sup>-1</sup>, TB  $1.5$  mg.l<sup>-1</sup>, and ALB  $3.7$  mg.l<sup>-1</sup> ( $p = 1.0$ ).

### Histopathological assessment

The liver sections showed considerable hepatocyte necrosis, hyaline degeneration, fatty modification, lymphocyte and Kupffer cell infiltration, and quantitative scoring to evaluate acute hepatic injury (Fig. 2). According to The French Metavir Cooperative Study Group [20] methodology, liver pathology is assessed using a scoring system where 0 denotes no cell damage

**Table 3.** Histological injury score of liver under different treatments.

Group**	Injury of score*			
	Fatty degeneration	Inflammation	Necrosis	Cell swelling
C	0	0	0	1
MP	0	1	1	1
S	1	1	0	1
E	0	1	0	0
E/MP <sup>a</sup>	1	0	0	3
S/MP	0	1	0	1
E/S/MP	1	1	0	0

\*: Livers were scored for hepatic injury via light microscopy with score 0: no visible cell damage; score 1: focal hepatocyte damage on less than 25% of the tissue; score 2: focal hepatocyte damage on 25%–50% of the tissue; score 3: extensive, but focal, hepatocyte lesions; score 4: global hepatocyte necrosis.

\*\* : C: Control received only distilled water and food; MP:MP at 40-mg/kg body weight; S: Silymarin at 50-mg/kg body weight; E: *E. fragilis* extract 30-mg/kg body weight; E/MP: *E. fragilis* extract 30 mg/kg and MP 40 mg/kg; S/MP: Silymarin 50 mg/kg and MP 40 mg/kg; E/S/MP: *E. fragilis* extract 15 mg/kg; silymarin 25 mg/kg, and MP 40 mg/kg.

a: mitosis has been detected.

and 1 represents less than 25% localized hepatocyte damage in the tissue [21].

Table 3 and Figure 2 display the histological findings of the current investigation on hepatic tissue evaluation. Hepatocyte necrosis, swelling and inflammation of cells, and fatty degeneration were among the morphological alterations analyzed and evaluated. The three most noticeable changes were fatty degeneration, inflammation, and cell swelling in that order. Changes in cell edema that were most visible were seen in group E/MP. Except for group E/S/MP, inflammation was most evident in these groups. MP-treated rats showed slight hepatic morphological changes, such as necrosis, cell edema, and inflammation, compared to the control group's normal liver tissues. Compared to a standard control, the injection of Alenda caused minor cell edema and inflammation. It has been shown that co-administration of Alenda and MP was linked to more noticeable fatty degeneration and cell enlargement. On the other hand, the MP-induced necrosis improved in groups E/MP, S/MP, and E/S/MP. Interestingly, group E/MP showed signs of mitotic activity.

## DISCUSSION

Liver cirrhosis is a leading cause of death in hepatic disorders, posing a significant public health threat. Despite advances in medicine, there are no effective drugs for protecting the liver or improving its function. Hepatic illnesses include viral infections, malignancies, alcoholism, drug overdoses, and nonalcoholic fatty liver disease. Treatment-induced liver impairment can lead to treatment withdrawal, and liver transplantation is necessary for end-stage liver disease. Plant-based phytochemicals are crucial for finding safer and more productive pharmaceutical substitutes.

Children with acute lymphoblastic leukemia are frequently treated with chemotherapeutic drugs such as methotrexate and MP, which can be harmful to the liver.

Hepatoprotective substances, such as milk thistle extract silymarin and herbals, have been utilized to lessen adverse effects. Silymarin does not counteract the effects of chemotherapy and has been demonstrated to reduce liver damage significantly. The sole traditional medication approved for hepatoprotection in the United States, ursodeoxycholic acid, has been connected to mutagenesis, liver cell failure, and an elevated risk of hepatocellular carcinoma. Low survival rates, morbidity, and graft rejection are possible outcomes of hepatotoxic liver transplants. There is a growing market for safer, more potent hepatoprotective medications, with Ayurvedic botanicals leading the way. The development of hepatoprotective medications that are well-tolerated, affordable, and safe is now undertaken.

*Ephedra fragilis*, a member of the *Ephedra* genus, has been used in traditional Chinese medicine for over 5,000 years for its anti-inflammatory, anti-invasive, anti-angiogenic, antimicrobial, neuroprotective, hepatoprotective, and antioxidant properties, primarily due to flavonoids, alkaloids, and phenolic acids [22]. This being the case, research has been proposed to investigate the potential benefits of *E. pachyclada* extract, which has been shown to possess liver-protective properties against CCl<sub>4</sub> liver inflammation [15]. The most common biochemical markers of liver damage are SGOT, SGPT, and ALP plasma levels.

The present investigation examines the course of liver damage resulting from repeated MP administrations and the potential hepatoprotective effect of concurrent consumption of *E. fragilis* extract compared to silymarin hepatoprotectant.

Both Wagners and Dragendorff yield positive scores for alkaloids. Moreover, saponins and tannins produce positive results for the qualitative phytochemical investigation. These findings conform to the literature [11,23]. The *E. fragilis* ethanol:water (33%) extract had a total phenolic content of 46.7 ± 1.7 mg GE per gram of dry extract (mean ± SEM). Compared to 13 mg, EAG/g previously reported in Algeria by Djahra *et al.* [10] 32.7 by Guenaou *et al.* [22], in Morocco, and 30.5 by Kmail *et al.* [24]. The flavonoid content was 76.6 ± 1.3 mg QE per gram of dried extract, which is higher than previously reported by Guenaou *et al.* [22], 10.5 mgQE/g. A higher value can be attributed to several factors such as time of collection, part used, species, soil content, solvent, and exact extraction method. DPPH Free Radical Scavenging IC<sub>50</sub> of 0.126 mg.ml<sup>-1</sup> indicates intense antioxidant activity, compared to 0.9 mg.ml<sup>-1</sup> reported by Djahra *et al.* [10] and reasonably comparable to ethyl acetate fraction extract accomplished by Guenaou *et al.* [22] that is 0.116 mg.ml<sup>-1</sup>.

The hydroalcoholic extract of *E. fragilis* successfully decreased the increase in plasma enzyme activity brought on by MP in the case of ALP, only besides a significant effect on TB levels. At the same time, there has been no considerable amelioration in SGPT or SGPT. They are indicating weak effectiveness in decreasing MP-induced liver damage. Increases in the enzymes, as mentioned earlier, may be a sign of hepatobiliary network injury and inflammation. Finding specific patterns at aberrant levels aids in locating the damage's origin. The hepatocellular disease is indicated by elevations in SGPT and SGOT that are not in line with ALP.

A sensitive indicator of hepatic injury is serum aminotransferase activity, which can change enzyme leakage due to hepatocyte transport activity and membrane permeability [25]. Hepatic tissue membranes sustain severe damage during MP treatment, exposing the bloodstream to SGOT and SGPT. Enzymatic defenses against reactive free radicals, such as catalase and superoxide dismutase, are present in hepatic cells [26]. Rats given MP showed signs of hepatotoxicity, as seen by elevated SGOT, SGPT, TB, and ALP plasma levels. This finding is consistent with previous studies that found MP-caused hepatotoxicity in drug-treated rats due to a considerable rise in aminotransferase levels [27]. These results are pretty different or disagree with the previous study that evaluated *E. Alata* against deltamethrin pesticide liver damage by Djahra *et al.* [10], where liver enzymes were reduced, and the extract shows an effect SGPT, SGOT, and ALP.

Hepatoprotective medications can prevent high ALP levels that are linked to drug-induced cholestasis. The amelioration effect against ALP levels when using *E. fragilis* in conjunction with MP indicates its probable selective effect against drug-induced cholestasis [28]. Drug-induced liver injury is a severe adverse reaction to drugs and xenobiotics, primarily affecting the liver. It can lead to cholestatic damage and is more common in older patients. There is a genetic predisposition to toxic cholestasis, and bile duct injury is a significant prognostic finding in this situation. *E. fragilis* could be a suitable protective element if additional study is done to investigate its effect in conjunction with some of these medicines.

An endogenous anion called bilirubin helps the liver produce and excrete bile. The liver gets hyperbilirubinemia if it cannot eliminate bilirubin because of hepatocyte damage. Total bilirubin levels indicate the degree of liver disease. MP treatment results in mixed hepatotoxicity and markedly lowered liver activity of catalase and superoxide dismutase.

The investigation's findings show that *E. fragilis* extract may influence catalase and superoxide dismutase activities, indicating that the hepatoprotective action of Alenda extract may entail the modulation of hepatic antioxidant enzymes. This may suggest further targeted and focused study to determine how this impact, represented in biochemical output, is produced. Due to their ability to conjugate to decreased GSH, antioxidant enzymes are essential for detoxifying xenobiotics. Since free radicals affect the body's different enzyme functions, they are a significant source of nonenzymatically caused lipid peroxidation in MP ingestion, and they may be connected to enzyme-induced lipid peroxidation [6]. Thus, when compared to normal rats, treated rats with MP may benefit from an *E. fragilis* hydroalcoholic extract to possibly counteract reduced superoxide dismutase and catalase activities. The possible hepatoprotective action of *E. fragilis* extract is not significantly different from that of silymarin, a well-known standard hepatoprotectant, concerning ALP levels.

However, compared to *E. fragilis* alone, the combination of *E. fragilis* and silymarin significantly improves and is less than that of the silymarin-treated group. This effect is reflected by the combination dose used. More investigation is needed to figure out the best combination from Alenda alone or combined with silymarin to get better benefits from the possible

synergistic effect that might result. The study found that Silymarin reduces cell edema and inflammation, while *E. fragilis* extract reduces inflammation, necrosis, and liver lesions caused by MP. The extract may inhibit MP-triggered oxidative liver damage by preventing cells from being immediately affected by MP and decreasing damage caused by slow inflammation. This result aligns with previous studies showing *E. fragilis* extract's ability to shield rat liver from oxidative damage caused by deltamethrin [11]. The results showed significant decreases in histopathological analysis, ALB, SGOT, SGPT, and ALP levels. According to Saidia *et al.* [29], *E. alata* Aqueous is effective in preventing and treating a variety of organ toxicities.

In summary, the improvement in ALP levels suggests that it has a likely selective impact on drug-induced cholestasis. *Ephedra fragilis* extract may influence catalase and superoxide dismutase activities, suggesting that the hepatoprotective action of Alenda extract may involve modulating hepatic antioxidant enzymes. That is supported by DPPH Free Radical Scavenging IC<sub>50</sub> and high flavonoid content. *Ephedra fragilis* extract reduces inflammation, necrosis, and liver lesions caused by MP. The extract may inhibit MP-triggered oxidative liver damage by preventing cells from being immediately affected by MP and decreasing damage caused by slow inflammation.

## CONCLUSION

The hydroalcoholic extract of *E. fragilis* was tested for hepatoprotective properties. The extract's phytochemical composition ends with indications that alkaloids, tannins, and saponins will also get positive results. *Ephedra fragilis* extract has appreciated Free Radical Scavenging potential, substantial polyphenols, and remarkable flavonoid content. Furthermore, the data obtained indicate that the aerial portion is an aqueous extract of *E. fragilis* subsp. Alenda has a significant potential to be an anti-drug-induced cholestasis action via lowering ALP levels. These results provide biochemical support for the potential therapeutic use of *E. fragilis* in treating a few hepatic disorders, including drug-induced cholestasis.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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Ethical approval details is given in the 'Experiment section'.

## DATA AVAILABILITY

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## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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