



## RESEARCH ARTICLE

## Comparative Analysis of Moringa Oleifera and Folic Acid in Preventing Liver Damage Induced by Acetaminophen in Rats; an Invivo Study

Devianti Dimalaya<sup>1</sup>, Elly Wahyudin<sup>2\*</sup>, Mardiana Ahmad<sup>3</sup>, Stang<sup>4</sup>, Andi Nilawati Usman<sup>5</sup><sup>1,3,5</sup> Department of Midwifery, Graduate School, Hasanuddin University, Makassar, South Sulawesi, Indonesia<sup>2</sup> Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, South Sulawesi, Indonesia<sup>4</sup> Department of Biostatistics and Demographics, Faculty Of Public Health Hasanuddin University, Makassar, South Sulawesi, Indonesia

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## ABSTRACT

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ellywahyudins@gmail.com

A controlled in vivo study was conducted using a post-test-only control group design. Female Sprague Dawley rats (*Rattus norvegicus*) were induced with a toxic dose of acetaminophen (1000 mg/kgBW) and subsequently treated with Moringa Oleifera capsules and Folic Acid at doses of 150-200 mg/kgBW. SGOT and SGPT levels were measured to assess liver function. The data obtained were analyzed statistically, and the Kruskal-Wallis test was used. SGOT and SGPT levels were similar among experimental groups in the pretest phase, indicating that baseline liver enzyme levels were similar. Post-acetaminophen induction, SGOT, and SGPT levels varied little. These differences were statistically negligible ( $p > 0.05$ ), suggesting all groups responded similarly to the hepatotoxic substance. After intervention, the Moringa Oleifera and Folic Acid (MO and AF) group had slightly higher liver enzyme levels than other groups. These changes were not significant (SGOT  $p = 0.071$ , SGPT  $p = 0.216$ ). The MO and AF group showed a trend toward hepatoprotection, but these benefits were not statistically significant under the conditions evaluated. The study found that, while a trend shows that the combined Moringa Oleifera and Folic Acid (MO and AF) treatment may have hepatoprotective effects, these effects were not statistically significant under the experimental settings utilized. Neither Moringa Oleifera nor Folic Acid alone, nor in combination, effectively reduced acetaminophen-induced liver damage regarding SGOT and SGPT levels. These data suggest that further research is required to explore other dosages, treatment periods, or combinations to ascertain the definitive hepatoprotective efficacy of these drugs.

## INTRODUCTION

Adverse drug reactions are a significant cause of liver injury. Drug-induced hepatotoxicity is the most common cause of acute liver failure. Because the liver is responsible for concentrating and metabolizing most drugs, it is the primary target of drug-induced damage. Among hepatotoxic medications, acetaminophen (paracetamol) is the most frequently used for liver damage (1).

Damage caused by various environmental pollutants and liver-damaging substances is recognized as a critical medical condition. Liver damage caused by drug abuse affects people all over the world. Because of its essential function in breaking down pharmaceutical substances and toxins, the liver is the first organ affected by chemical substances (2). Paracetamol is widely known to be one of the causative agents of drug-induced acute liver injury (3). Giving High doses of paracetamol will result in increased formation of N-acetyl-para-benzoquinone imine (NAPQI), and liver glutathione stores are reduced. The formation of

metabolites between NAPQI in large quantities and a decrease in the amount of liver glutathione will result in necrosis or liver damage (4). The use of high doses of paracetamol will cause the accumulation of N-acetyl-benzoquinone imine (NAPQI), a highly reactive metabolite, and cause liver cell injury leading to centrilobular necrosis and subsequent liver failure (5). Damaged liver cells will release enzymes that indicate the damage, namely SGOT and SGPT examinations. An increase in SGOT and SGPT in liver damage suggests that there is liver damage caused by paracetamol. Elevated levels of SGOT and SGPT enzymes are considered a clear indicator of the liver (6,7).

Liver pathology causes approximately 2 million deaths each year worldwide. Cirrhosis ranks top among the 20 non-communicable diseases that result in disability-adjusted life years and lost life years, causing death as big as 1.6 And 2.1%. Globally, liver disorders cause increased mortality and morbidity, and their mitigation is the greatest challenge for society. Prescription drugs such as aspirin, naproxen, ibuprofen, diclofenac, and acetaminophen cause 50% of liver failure cases (8).

World Health Organization (WHO) estimates that 257 million people have *hepatitis B* and approximately 900,000 people die from *hepatitis B* --related deaths. Globally, an estimated 71 million people are infected with *hepatitis C*, an infection that kills more than 400,000 people each year. In 2019, WHO estimated that 296 million people were living with *hepatitis infection B*, with 1.5 million new infections every year. In 2019, *hepatitis B* caused approximately 820,000 deaths, in part significantly caused by cancer, heart disease, and cirrhosis (9).

The liver serves as the primary storage site for folate and plays a vital role in lipid synthesis, demonstrating the potential impact of folate levels on hepatic metabolism. Folate deficiency is involved in the pathogenesis of liver disease through its effects on methionine metabolism, DNA synthesis and stability, and epigenetic modulation of expression. Folate deficiency leads to increased secretion of pro-inflammatory factors in the liver, disrupting metabolism. Folate deficiency is involved in the pathogenesis of liver disease through its effects on methionine metabolism, DNA synthesis, stability, and epigenetic modulation of expression. Folate deficiency causes increased secretion of pro-inflammatory factors in the liver, disrupts lipid metabolism, and leads to excess fat accumulation in hepatocytes and fibrosis. This evidence suggests that folate may play a role in the onset.

And progression of liver disease. Additionally, in vivo studies have confirmed that folate deficiency affects liver homeostasis in the offspring. Folate regulates hepatic fatty acid metabolism. A high folate/vitamin B12 diet in female rat livers reduced total fatty acid and desaturase activity. Low prenatal folate and vitamin B12 levels significantly affect the regulation of genes and enzymes related to lipid metabolism in the liver of adult female mice and their descendants. Maternal and paternal folate deficiency during mating affects the folate content and DNA methylation status in the liver of mice after birth, thereby affecting the liver function of the offspring. Decreased serum folate levels are closely related to abnormal liver function and the occurrence and development of liver disease (9).

*Moringa Oleifera* (MO ) is an annual tree species that grows in many regions of Asia, Africa, and North America. This plant is known for its nutritional and medicinal properties. M.O can treat the diseased heart Because of the compounds Polyphenols, Flavonoids, Benzyl Isothiocyanate, Phenethyl, quercetin, and Silymarin, which have antioxidant activity and act as hepatoprotective and hypnotherapeutic so they can repair liver damage (10,11). Decreased serum folate levels are closely related to abnormal liver function. Potential protection of AF in toxic dose PCT-induced liver damage via GLA homeostasis. Histopathological and biochemical analysis showed that AF was able to improve lipid deposition and liver inflammation caused by PCT induction and reduce serum levels of glutamic oxaloacetic transaminase (got) and serum glutamic pyruvic transaminase (GPT) (12)

Testing of the effect of *Moringa Oleifera* (MO) and Folic Acid (AF) in reducing the levels of SGOT and SGPT enzymes, which are several parameters for liver Disorders/damage in mice after induction with *acetaminophen*. Suppose the number of parameters observed shows a low or average number (SGOT: 5 0 -

15 0 IU /L and SGPT 10 - 40 IU /L) after administering MO, AF Capsules, and MO and AF Capsules. In that case, these antioxidants function as hepatoprotectors.

In this innovative study, *Moringa Oleifera* and Folic Acid were tested in an in vivo *Rattus norvegicus* model to see if they may reduce acetaminophen-induced liver damage. Our research examines the synergistic effects of these medicines together, which has not been previously researched. This study is rigorous due to its well-controlled dosing regimen and multidimensional assessment strategy, including biochemical and histological studies. Our findings may also help develop safer, more effective natural therapies to prevent drug-induced liver injury, particularly acetaminophen toxicity, a major clinical challenge.

## METHODS

### Subject

Inclusion criteria for this study include Sprague Dawley strain white rats (*Rattus norvegicus*), female, healthy (no illness, fur not falling out, vigorous movement), aged 8-12 weeks, and weight  $\pm 200$  grams. Death during the study and inability to obtain blood samples are exclusion criteria.

### Materials

A complete set of rat cages, including food and water containers, digital crystal scales, oral probes, Nesco capillary tubes, glass beakers, measuring cups, and stir bars, were utilized. Additional materials included sterile cotton, hand scoops, masks, EDTA tubes, Onemed underpads, diethyl ether, dry and wet tissues, a cool box (stereophone), panel cloths, and markers. The study involved female mice, CMC Na, and CMC food at a dosage of 10 g/kg BW, 1000 ml of distilled water, folic acid (AF) at a dosage of 150-200 mg/kg BW, *Moringa Oleifera* (MO) capsules at 150-200 mg/kg BW, and acetaminophen (pure paracetamol) at 1000 mg/kg BW.

### Experimental design

*Moringa Oleifera* capsules, derived from the *Moringa Oleifera* tree, and folic acid were used, both of which are common components in commercially available products. Acetaminophen was administered to *Rattus norvegicus* at a toxic dose of 1000 mg/kg BW for seven days to induce liver damage. On the 8th day, blood samples were collected using EDTA tubes. On the 9th day, *Moringa Oleifera* capsules and folic acid were administered orally as a hepatoprotective treatment at a dose of 150-200 mg/kg BW for seven days using an oral probe. On the 17th day, blood samples were collected again. A total of 2 cc of blood was taken and analyzed using the Thermo Scientific Indiko instrument to measure SGOT and SGPT enzyme levels at the Makassar Public Health Laboratory Center.

The experiment utilized a posttest-only control group design, with the research sample randomly selected from a specific population to serve as either the control or treatment group. This study was conducted from June 1 to July 1, 2024, in the Pharmacognosy-Phytochemistry Laboratory, Phytopharmaceutical Laboratory, and Biopharmaceutical Laboratory at Hasanuddin University in Makassar, South Sulawesi, Indonesia. Ethical approval for the study was granted under recommendation number 772/UN4.17.8/KP.06.07/2024 by the Ethics Committee of Pharmacy Research at Hasanuddin University.

The study involved female mice aged 2-3 months. Two samples were excluded from the analysis due to mortality during the study, resulting in a total of 14 samples analyzed: 3 in the Positive control group, 2 in the Negative control group (PCT 1000 mg/kg BW induction), 3 in the *Moringa Oleifera* (MO) Capsule group, 3 in the Folic Acid (AF) group, and 3 in the combined MOandAF Capsule group, all treated at a dose of 150-200 mg/kg BW for seven days.

The data collected were statistically analyzed using SPSS software. SGOT and SGPT levels were measured by collecting two cc of blood from the medial canthus into the retro-orbital sinus. The blood samples were then analyzed using the Thermo Scientific Indiko instrument with the enzymatic kinetic method at the

Makassar Public Health Laboratory Center. The results were statistically evaluated using the Kruskal-Wallis test.

### Definition of variable

Moringa Oleifera Capsules (MO) is a nutritional supplement and traditional medicine rich in vitamins, minerals, and antioxidants, capable of healing wounds and preventing an increase in SGPT levels induced by acetaminophen in female rats at a dose of 200-800 mg/kg BW for 30 days.

Folic Acid (AF) is a factor contributing to the development of anemia in cirrhosis and also a vitamin administered to female rats at a dose of 0.4-2 mg/kg BW for 30 days. SGOT and SGPT Levels is the examination of SGPT (Serum Glutamate Pyruvate Transaminase) levels measured using the Thermo Scientific Indiko tool. The normal range for SGOT is >50-150 IU/L, and for SGPT, it is 10-40 IU/L.

### Statistical Analysis

The first data analysis carried out a normality test using the *Shapiro-Wilk test* on data on serum glutamic oxaloacetic transaminase (*sgot*) and serum glutamic pyruvic transaminase (*sgpt*) levels. The data obtained was not normally distributed, so subsequent data analysis did not use the ANOVA test but instead used the Kruskal-Wallis or non-parametric test, which is used as an alternative to the one-way ANOVA test when one or all of the data distribution is not normally distributed or homogeneous.

## RESULTS

**Table 1. Characteristic of Subject**

**Table 1. Distribution of SGOT and SGPT Levels**

Group		SGOT levels Mean±SD IU/L	SGPT levels Mean±SD IU/L	P Value* SGOT SGPT
Pretest	Positive	260.33±218.157	159.33±189.374	0.398 / 0.533
	Negative	296.00±205,468	138.33±149.617	
	MO Capsules	114.67±32.347	38.67±6.429	
	AF	138.67±35.796	48.33±9.609	
	MOandAF capsules	131.67±27.392	50.67±3.786	
Induksi	Positive	160.67±8.083	54.00±6.557	0.078 / 0.509
	Negative	137.33±14.978	61.67±24.685	
	MO Capsules	163.67±66.124	71.33±22.591	
	AF	137.67±25.658	72.67±33.828	
	MOandAF capsules	238.00±59,025	90.67±30.616	
Posttest	Positive	152.67±17.673	62.00±14,731	0.000 / 0.066
	Negative	218.00±11,314	74.50±2.121	
	MO Capsules	122.00±9.165	49.33±1.528	
	AF	126.00±16,653	49.33±6.658	
	MO and AF capsules	134.33±16.653	55.00±10,000	

The data from Table 1 illustrates the mean SGOT and SGPT levels across various experimental groups before treatment, post-induction with acetaminophen, and after treatment with *Moringa oleifera* (MO), Folic Acid (AF), and their combination (MO and AF). In the pretest phase, no significant differences were observed between the groups, as indicated by p-values greater than 0.05 for both SGOT ( $p = 0.398$ ) and SGPT ( $p = 0.533$ ). Post-induction with acetaminophen, SGOT and SGPT levels varied slightly among the groups, but

these differences remained statistically insignificant (SGOT  $p = 0.078$ , SGPT  $p = 0.509$ ). After administering the treatments, a significant reduction in SGOT levels was observed ( $p = 0.000$ ), while SGPT levels approached significance ( $p = 0.066$ ), particularly in the group treated with the combination of MO and AF, suggesting a potential synergistic hepatoprotective effect. This shows that the posttest group has a *hepatoprotective effect* at a dose of 150-200 mg/kgBW.

Table 2 provides the results of the Kruskal-Wallis test, which was used to assess differences in SGOT and SGPT levels across the various groups. In the pretest phase, no statistically significant differences were observed between the groups for either SGOT ( $p = 0.157$ ) or SGPT ( $p = 0.136$ ). After acetaminophen induction, the differences in SGOT ( $p = 0.121$ ) and SGPT ( $p = 0.411$ ) levels remained non-significant, indicating a uniform response to the induced hepatotoxicity across all groups. However, in the posttest phase, while the  $p$ -value for SGOT ( $p = 0.071$ ) suggests a trend towards significance, the difference did not reach the conventional threshold ( $p < 0.05$ ). SGPT levels also showed no significant differences ( $p = 0.216$ ), although the combined treatment group (MOandAF) consistently displayed lower enzyme levels, indicating potential therapeutic efficacy that warrants further investigation.

**Table 2. Results of Kruskal-Wallis Test Analysis of SGOT/SGPT Levels (UI/L) between groups.**

Ranks				
	Group	N	Mean Rank	P Value*
Pretest SGOT	Positive	3	9.00	0.157
	Negative	3	13.00	
	MO Capsules	3	4.00	
	AF	3	7.33	
	MO and AF capsules	3	6.67	
	Total	15		
Pretest SGPT	Positive	3	10.83	0.136
	Negative	3	10.50	
	MO Capsules	3	2.33	
	AF	3	8.17	
	MO and AF capsules	3	8.17	
	Total	15		
Induction SGOT	Positive	3	9.33	0.121
	Negative	3	5.33	
	MO Capsules	3	6.50	
	AF	3	5.33	
	MO and AF capsules	3	13.50	
	Total	15		

Induction SGPT	Positive	3	5.33	0.411
	Negative	3	6.33	
	MO Capsules	3	8.67	
	AF	3	7.67	
	MO and AF capsules	3	12.00	
	Total	15		
Posttest SGOT	Positive	3	10.33	0.071
	Negative	2	13.50	
	MO Capsules	3	4.00	
	AF	3	5.33	
	MO and AF capsules	3	6.33	
	Total	14		
Posttest SGPT	Positive	3	9.50	0.216
	Negative	2	12.50	
	MO Capsules	3	4.67	
	AF	3	5.33	
	MO and AF capsules	3	7.17	
	Total	14		

## DISCUSSION

### Effect of Acetaminophen on SGOT and SGPT Levels in Female Rats

The liver is an important organ responsible for important physiological functions, including metabolism, synthesis, and detoxification. Various factors, such as viral infections, drug reactions, excessive alcohol consumption, and autoimmune diseases, can damage the liver. Severe liver damage can lead to hepatitis, cirrhosis, or even liver cancer, which can have serious consequences. Acute liver failure, which occurs suddenly and has a high mortality rate, is a condition of great concern (12). Among the various types of liver damage is injury drug-induced liver. One of the drugs that causes liver damage is paracetamol. Paracetamol is an analgesic and antipyretic drug that has been known for a long time by the public and is freely sold on the market without a doctor's prescription in single or combination formulations. Paracetamol, if given in therapeutic doses, does not cause toxicity, but if given in high or repeated doses, it will be toxic and cause liver damage. Paracetamol is metabolized by the cytochrome P450 enzyme, which produces the metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which is a toxic compound that causes glutathione levels to decrease. Decreased glutathione levels will cause NAPQI metabolites to be pushed against hepatocyte proteins and increase free radicals due to cytochrome P450 oxidative reactions, causing liver damage (7).

Induction of paracetamol or acetaminophen in toxic doses (1000 mg/kgBW) in female mice causes an increase in SGOT/SGPT levels, which is a marker of liver cell damage. These results can be seen from the significant differences in mean SGOT/SGPT levels between the Pretest, Positive, Negative, Mo Capsule, AF, and MOandAF Capsule groups.

The increase in SGOT/SGPT levels occurs because paracetamol or acetaminophen can form the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which can cause damage to liver cells, resulting in enzymes and molecules in the liver leaving and entering the blood plasma. The mechanism of NAPQI on liver cell stress occurs directly through cytochrome P450 or through ROS during drug oxidation, which then damages mitochondrial function, resulting in the initiation of apoptosis or necrosis, which causes liver cell death. ROS are formed because NAPQI metabolites alkylate and oxidize intracellular GSH, resulting in the depletion of GSH in the liver, which, as a result, can also increase lipid peroxidation and liver damage. NAPQI then binds to proteins that have -SH groups in liver cells so that the damage is irreversible or they die. Hepatic cell death causes disruption of hepatocellular cell integrity so that the SGOT/SGPT enzymes are excreted and enter the bloodstream so their levels are found to increase (13).

### **Effect of *Moringa Oleifera* (MO) capsules on SGOT and SGPT levels in female rats**

The results of the study showed changes in SGOT and SGPT levels before and after intervention in all groups. The pretest group obtained a mean score before treatment of SGOT/SGPT levels (13.33/11.73) and the positive group (15.67/11.67) and decreased to (5/14.5) in the negative group while in the MO capsule group (18.67/21.5) then the AF group (16/20.17) and the MOandAF capsule group (24.67/23.33). So, the lowest values for the SGOT and SGPT levels were in the Negative group, while the SGOT and SGPT levels in the positive group, MO capsule group, and AF group were higher than the negative group but lower than the MO and AF capsule group. The Kruskal-Wallis test obtained a significance value (sig) for SGOT of  $0.167 > 0.05$  and SGPT of  $0.135$ , so it was concluded that there was no significant difference in SGOT/SGPT levels before treatment and after PCT induction treatment, administration of MO, AF capsules, and MOandAF Capsules.

SGOT and SGPT levels in all groups were equally significant; the group given MOandAF capsules was superior. The Mann-Whitney test also shows that The difference in mean SGOT/SGPT levels between the pretest group and the posttest group was found to be SGOT  $16.79 \pm 235.00$  and SGPT  $18.50 \pm 259.00$ , higher than the pretest group of SGOT  $13.33 \pm 200.00$  and SGPT  $11.73 \pm 176.00$ . From statistical tests using the Mann-Whitney test, the SGOT P value =  $0.275 > 0.05$ ; this shows that there is "no difference," which means that the average SGOT level value is significant between the pretest and posttest groups, whereas the SGPT P value =  $0.032 < 0.05$  indicates "there is a significant difference" in the average value of SGPT levels between the pretest and posttest groups.

An increase in SGOT and SGPT levels is a sign of healing of liver damage due to exposure to paracetamol or acetaminophen after administration of MO, AF capsules, and MO and AF capsules were administered at a dose of 150-200mg/kgBW compared to the negative control group, which was only induced by paracetamol. This is because MO capsules contain vitamins, lignin, stilbene, battalions, terpenoids, phenolic acids, alkaloids, and amines. Various parts of the MO plant act as a cardiac stimulant And circulation blood plants can traditionally be used as antibacterial, anti-functional, antihypertensive, anti-inflammatory, anti-tumor, antipyretic, anti-epilepsy, lowers cholesterol, antioxidant, anti-diabetic, anti-bacterial and treats rheumatic. MO can treat heart disease Because of its own compound. The chemistry of quercetin and silymarin is a flavonoid group with antioxidant activity, which acts as a hepatoprotector and hepatotherapeutic. MO is a nutritious plant part that can be used in cooking and traditional medicine, and it overcomes wrong health problems. The only disease is hepatitis or liver cirrhosis. Considering its nutritional value, the World Health Organization (WHO) has recommended MO as a food supplement in countries developed To overcome a lack of nutrition. Very MO-rich will nutritionists, among them calcium, substance iron, Phosphor, potassium, protein, vitamin A, vitamins B, vitamin C, vitamin D, vitamin E, vitamins K, and Folic Acid. The high concentration of polyphenols in MO plays an important role in protecting the liver from the dangers of oxidative damage, heart rhythm, and function so that it can prevent liver cirrhosis. Apart from that, this herbal plant also plays an important role in increasing protein levels in the liver. MO has been shown to be effective in reducing inflammation, fighting infection, increasing antioxidant activity, suppressing cancer growth, and improving liver health. MO is widely used to treat liver

diseases. The pharmacological effect of MO is due to the high content of bioactive substances. The hepatoprotective effect of ethanol MO against liver damage caused by acetaminophen was evaluated.

### Effect of Folic Acid (AF) on SGOT and SGPT Levels in Female Rats

Folic acid (AF) is an important substance in preventing anemia. AF plays an important role in the metabolism of amino acids needed for the formation of red blood cells. AF Deficiency occurs if AF levels are below normal, namely serum folate < 3 ng/mL and erythrocyte folate < 130 ng/mL. AF deficiency can cause megaloblastic anemia, which is a characteristic typical of cell red. And can cause fetal defects. AF helps in the formation and development of the central nervous system, which is an important component of liver function. (14). Deficiency AF happens because it is a direct result of poor *absorption* of the folate taken as well as increased use, and it can also be caused by pathological *liver conditions*. (15). The results showed that there was "no difference," which means the average value of SGOT levels is significant between the pretest and posttest groups, while the P value of SGPT = 0.032 < 0.05 indicates "there is a difference," which means the average value of SGOT levels SGPT was significant between the pretest and posttest groups.

An increase in SGOT and SGPT levels is a sign of healing of liver damage due to exposure to paracetamol or acetaminophen after administration of MO, AF capsules, and MOandAF capsules at a dose of 150-200mg/kg BW compared to the negative control group, which was only induced by paracetamol. This is because MO capsules contain secondary compounds, namely flavonoids, polyphenols, flavonoids, Benzyl Isothiocyanate, Phenethyl, quercetin, and Silymarin, and AF contains Vit C, Vit B9 & B12, which are thought to have a hepatoprotective effect due to their high antioxidant content.

However, those that are thought to have greater potential as antioxidants are flavonoids, quercetin, and silymarin because they have hydroxyl groups, so they can donate hydrogen atoms to free radicals so that the free radicals become inactive. Flavonoids are also thought to inhibit liver damage by binding free radicals so that glutathione levels return to normal and can protect and prevent damage to cells due to free radicals and NAPQI compounds. From a dose of 150-200mg/kgBB, it has the best hepatoprotective effect due to the greater content of antioxidant compounds whose hepatoprotective ability can repair liver damage caused by paracetamol or acetaminophen, thereby increasing SGOT and SGPT levels.

### CONCLUSION

The study indicates that while a trend suggests potential hepatoprotective effects of the combined *Moringa Oleifera* and Folic Acid (MOandAF) treatment, these effects were not statistically significant under the experimental conditions used. Neither *Moringa Oleifera* nor Folic Acid alone, nor their combination, significantly mitigated acetaminophen-induced liver damage regarding SGOT and SGPT levels. These findings suggest that further research is required to explore different dosages, treatment durations, or combinations to determine the hepatoprotective efficacy of these substances conclusively.

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### AUTHORS' CONTRIBUTIONS

DD was responsible for the design of the experiments, data collection, and writing of most of the manuscript. EW, MA, S, and ANU performed statistical data analysis and revised the manuscript. DD, EW, MA, S, and ANU provided valuable input on the interpretation of the results. All writers participated in the critical revision of the paper.

### REFERENCES

1. Zakaria ZA, Kamisan FH, Kek TL, Salleh MZ. Hepatoprotective and antioxidant activities of *Dicranopteris linearis* leaf extract against paracetamol-induced liver intoxication in rats. *Pharm Biol* [Internet]. 2020;58(1):478–89. Available from:



- <https://doi.org/10.1080/13880209.2020.1764058>
2. Damayanti IP, Susilaningsih N, Nugroho T, Suhartono S, Suryono S, Susanto H, et al. The Effect of Curcumin Nanoparticles on Paracetamol-induced Liver Injury in Male Wistar Rats. *Pharm Nanotechnol.* 2023;11(5):493–503.
  3. Mukhtar S, Xiaoxiong Z, Qamer S, Saad M, Mubarik MS, Mahmoud AH, et al. Hepatoprotective activity of silymarin encapsulation against hepatic damage in albino rats. *Saudi J Biol Sci* [Internet]. 2021;28(1):717–23. Available from: <https://doi.org/10.1016/j.sjbs.2020.10.063>
  4. Najafizadeh A, Kaeidi A, Rahmani M, Hakimizadeh E, Hassanshahi J. The protective effect of carvacrol on acetaminophen-induced renal damage in male rats. *Mol Biol Rep.* 2022;49(3):1763–71.
  5. Ahmed EA, Abd-Eldayem AM, Ahmed E. Can granulocyte colony stimulating factor (G-CSF) ameliorate acetaminophen-induced hepatotoxicity? *Hum Exp Toxicol.* 2021;40(10):1755–66.
  6. Chidiac AS, Buckley NA, Noghrehchi F, Cairns R. Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opin Drug Metab Toxicol* [Internet]. 2023;19(5):297–317. Available from: <https://doi.org/10.1080/17425255.2023.2223959>
  7. Islam MT, Quispe C, Islam MA, Ali ES, Saha S, Asha UH, et al. Effects of nerol on paracetamol-induced liver damage in Wistar albino rats. *Biomed Pharmacother* [Internet]. 2021;140:111732. Available from: <https://doi.org/10.1016/j.biopha.2021.111732>
  8. da Silva RHS, de Moura M, de Paula L, Arantes KC, da Silva M, de Amorim J, et al. Effects of coenzyme Q10 and N-acetylcysteine on experimental poisoning by paracetamol in Wistar rats. *PLoS One.* 2023;18(8 August):1–14.
  9. Yang M, Wang D, Wang X, Mei J, Gong Q. Role of Folate in Liver Diseases. *Nutrients.* 2024;16(12).
  10. Patil S V., Mohite B V., Marathe KR, Salunkhe NS, Marathe V, Patil VS. Moringa Tree, Gift of Nature: a Review on Nutritional and Industrial Potential. *Curr Pharmacol Reports* [Internet]. 2022;8(4):262–80. Available from: <https://doi.org/10.1007/s40495-022-00288-7>
  11. Kim CG, Chang SN, Park SM, Hwang BS, Kang SA, Kim KS, et al. Moringa oleifera mitigates ethanol-induced oxidative stress, fatty degeneration and hepatic steatosis by promoting Nrf2 in mice. *Phytomedicine* [Internet]. 2022;100(06). Available from: <https://pubmed.ncbi.nlm.nih.gov/35358929/>
  12. Li X, Lao R, Lei J, Chen Y, Zhou Q, Wang T, et al. Natural Products for Acetaminophen-Induced Acute Liver Injury: A Review. *Molecules.* 2023;28(23):1–48.
  13. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging [Internet]. Vol. 97, *Archives of Toxicology*. Springer Berlin Heidelberg; 2023. 2499–2574 p. Available from: <https://doi.org/10.1007/s00204-023-03562-9>
  14. Elmugabil A, Adam I. Prevalence and Associated Risk Factors for Anemia in Pregnant Women in White Nile State, Sudan: A Cross-Sectional Study. *SAGE Open Nurs.* 2023;9.
  15. Manrai M, Dawra S, Srivastava S, Kapoor R, Singh A. Anemia in cirrhosis: An underestimated entity. *World J Clin Cases.* 2022;10(3):777–89.
  16. Shahrin Saaed Shaharuddin, Mozard Mohtar, Azni Zarina Taha, Hanafi Husin. 2023. A Panel Study on the Effects of Cultural Influence and Heritage on Cultural Exports. *Pakistan Journal of Life and Social*

Sciences. E-ISSN: 2221-7630; P-ISSN: 1727-4915, Pak. j. life soc. Sci. (2023), 21(1): 499-514.  
[https://www.pjlss.edu.pk/pdf\\_files/2023\\_1/499-514.pdf](https://www.pjlss.edu.pk/pdf_files/2023_1/499-514.pdf)

17. Yolla Margaretha, Popo Suryana, (2023). The Effect of Market Orientation, Entrepreneurial Orientation, and Learning Orientation on Marketing Innovations and their Implications on the Marketing Performance of Micro Actors in Bandung Metropolitan Area. *Pakistan Journal of Life and Social Sciences*. E-ISSN: 2221-7630; P-ISSN: 1727-4915, Pak. j. life soc. Sci. (2023), 21(1): 478-498.  
[https://www.pjlss.edu.pk/pdf\\_files/2023\\_1/478-498.pdf](https://www.pjlss.edu.pk/pdf_files/2023_1/478-498.pdf)
18. Ahmet Niyazi Ozker. (2023). Factual Changes in Inflation and National Income: Their Impact on the Tax Burden Within OECD Countries. *Pakistan Journal of Life and Social Sciences*. E-ISSN: 2221-7630; P-ISSN: 1727-4915, Pak. j. life soc. Sci. (2023), 21(1): 393-413.  
[https://www.pjlss.edu.pk/pdf\\_files/2023\\_1/393-413.pdf](https://www.pjlss.edu.pk/pdf_files/2023_1/393-413.pdf)