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Hepatoprotective Effect of *Spondias pinnata* in Isoniazid-Rifampicin-Induced Toxicity in Wistar rats

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ABSTRACT

Anti-tubercular (TB) drugs can cause oxidative stress, leading to hepatotoxicity. Plant extracts have antioxidant effects that can reduce oxidative stress. One plant with a high antioxidant capacity is *Spondias pinnata*. This study attempts to demonstrate that *Spondias pinnata* leaf extract, at doses of 200, 400, and 600 mg/kg bw (body weight), can decrease oxidative stress in rats treated with isoniazid-rifampicin. Five treatment groups, normal control (K0), negative control (K1), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3) were included in this experiment. Male Wistar strain (8–12 weeks old) and weighed 200–220 g totalling 35 were used in this study. K1, P1, P2, and P3 rats were given isoniazid-rifampicin for 28 days. Rats in groups P1, P2, and P3 received *Spondias pinnata* leaf extract for 35 days (starting 7 days before induction). The ELISA method was used to assess oxidative damage through 8-hydroxy-2'-deoxyguanosin (8-OHdG) levels in rat liver tissue. In the rats' liver result, isoniazid and rifampicin cause DNA oxidative stress, as seen by an increase in 8-OHdG levels in K1 relative to K0. In comparison to K1, the administration of 200 mg/kg (P1), 400 mg/kg (P2), and 600 mg/kg (P3) of *Spondias pinnata* leaf extract lowered the amount of 8-OHdG in the liver of rats induced by isoniazid-rifampicin. This study shows that *Spondias pinnata* exhibited hepatoprotective effects, reducing 8-OHdG levels in isoniazid-rifampicin-induced rats.

Keywords: *Spondias pinnata*, Anti-tubercule, Isoniazid, Rifampicin, 8-OHdG

Introduction

Tuberculosis (TB) is a major global health concern, that affects approximately one-third of the world's population and is caused by the bacteria *Mycobacterium tuberculosis*.[1,2] The World Health Organization (WHO) claimed that each year, 1.5 million people die and 10 million new cases are reported, especially in low- and middle-income countries. Factors such as dense populations, high internal mobility, and limited healthcare access contribute to the disease's high prevalence.[3] According to estimates, there were 10.4 million new cases of tuberculosis (TB) in 2015. An anticipated 9.9 million persons had TB in 2020 and 10.6 million in 2021, with Southeast Asia and Africa having the most significant number and accounting for almost 70% of all TB cases. After two years of TB services being disrupted by the COVID-19 epidemic, WHO predicts a strong worldwide TB recovery in 2023.[4–7] More than 80% of the 10 million people who get active TB each year become better after treatment.[8] Untreated active TB has a high mortality rate of almost 50%. The World Health Organization's approved therapy can cure 59% to 95% of patients.[2]

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Treatments for TB typically include pyrazinamide, ethambutol, isoniazid, and rifampicin. It includes two months of intensive phase and four months of advanced phase.[6,9] The prolonged use of isoniazid (INH),[10] especially in combination with rifampicin, increases the risk of liver toxicity,[11–14] due to oxidative stress.[15,16] Anti-TB drugs cause hepatotoxicity by 1-36%.[14] Hepatotoxicity varies from asymptomatic to the occurrence of hepatocellular steatosis, centrilobular necrosis, and liver failure.[15,17] It is attributed to an imbalance in the liver's antioxidant defences, leading to increased free radicals and damage to cellular structures, including Deoxyribonucleic acid (DNA), altering signalling pathways with cell damage.[16,18–20] The oxidative stress caused by free radicals in hepatocytes that attack proteins, DNA, and cell membranes due to isoniazid and rifampicin metabolism has been linked to hepatotoxicity. N-acetyltransferase and hydrolase amide break down isoniazid in the liver to produce acetyl hydrazine (AcHz), isonicotinic acid, methylhydrazine (DiAcHz), mono acetyl hydrazine, acetyl isoniazid (AcINH), and hydrazine (Hz). Additionally, the cytochrome enzyme P450 2E1 (CYP2E1) oxidises AcHz and Hz into radical metabolites and transforms monoacetyl hydrazine into hazardous chemicals, resulting in liver damage and the production of reactive oxygen species (ROS). Hydrazine causes oxidative stress and mitochondrial malfunction, which results in energy depletion, cell dysfunction, and cell death by depleting the liver's stored amounts of glutathione (GSH).[21–23] When isoniazid and rifampicin are used together, the cytochrome P450 enzyme is stimulated, increasing the production of toxic by-products from hydrazine.[11,24–31] The nucleoside form of deoxyguanosine (8-hydroxy-2'-deoxyguanosin) or 8-hydroxyguanin (8-OHGua) is produced when reactive oxygen species interact with the guanine DNA base strand. 8-OHdG is a vital marker for assessing endogenous oxidative damage of DNA.[32]

Plants provide a natural source for treating various diseases through their antioxidant activity. Alkaloids, glycosides, flavonoids, fatty acids, saponins, and sterols are several substances in plant extracts

with potential therapeutic activities. Plant polyphenol chemicals are vital because they may scavenge free radicals by supplying hydroxyl groups.[33] Many plants can be used to treat liver problems[21], and hepatoprotective plants protect the liver.[17]

Spondias pinnata (LF) Kurz (Anacardiaceae), known as wild mango or hog plum, is primarily found in tropical and subtropical areas worldwide.[34] This plant contains several active components such as alkaloids, tannins, saponins, glycosides, terpenoids, flavonoids, polyphenols, essential oils, amino acids, carbohydrates, vitamins, and minerals,[35,36] which are believed to scavenge free radicals and mitigate oxidative damage.[35,36] These compounds can also treat liver toxicity.[37] This study aims to demonstrate the hepatoprotective efficacy of *Spondias pinnata* leaf extract in mitigating DNA oxidative stress in rats generated by isoniazid-rifampicin, as indicated by a reduction in hepatic 8-OHdG levels.

Materials and Methods

Materials

These include microtubes, refrigerators, ultrasonicators, centrifuges, Thermo Scientific Spectrophotometers, 0.5 mL Eppendorf tubes, 1.5 mL Eppendorf tubes, yellow tips, white tips, blue tips, ELISA Washers, ELISA readers, gloves and 8-OHdG.

Plant collection and Identification

Spondias pinnata leaves from Penglipuran Village in Bangli Regency, Bali Province, were collected in January 2024 and authenticated by a taxonomist (Ratna Yulianti) from Herbal Materia Medica Laboratory, Batu East Java, Indonesia as *Spondias pinnata* (L.f.) Kurz.

Plant preparation

After washing and removing debris, the leaves were allowed to air dry in a shaded area, away from direct sunlight. The leaves were thereafter heated in an oven at 40-50°C for 12 hours. The desiccated leaves were pulverized and filtered through a 40-mesh sieve, forming a fine powder (simplicia). Simplicia was divided and macerated with 70% ethanol at room temperature in a ratio of 1:5 for 24 hours. The extract was filtered to obtain filtrates and marc. The marc was extracted again two times to obtain three filtrates. Filtrates I, II, and III of each process were bulked and concentrated at 40°C in a rotating evaporator until a crude concentrated extract was produced.

Ethical approval and animals

Ethical approval was sought and obtained from Udayana University Faculty of Medicine's Research Ethics Commission Unit under number 1037/UN14.2.2.VII.14/LT/2024. *Rattus norvegicus* rats weighing 200–220 g and aged 8–12 weeks served as the test animals. The rats were acclimatised at the study site (The Integrated Biomedical Laboratory of Udayana University Denpasar's Faculty of Medicine) for one week, placed in a comfortable cage, fed standard feed, and unhindered access to drink water. In this investigation, the animals were exposed to a 12-hour light-dark cycle, with the temperature regulated at 25°C. Feeding was carried out every day from 08.00-09.00 am. The animals were weighed and only healthy rats were recruited for this study.

Experimental design

The animals were divided into five groups of 7 rats per group: normal control group (K0), negative control group (K1), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3). The treatment and dosing of the animals is shown in Table 1.

Ketamine HCl administered at a dose of 10% of 50 mg/kg bw was used to anesthetise the rats quickly and sterilely on the forty-third day. The animals were incised, and the liver was removed and samples were dried, weighed, and cleaned with phosphate buffer salt (PBS). The samples were stored in a freezer at -20°C and in a microtube with 500 µL of PBS.

Table 1: Treatment and dosing of Experimental Animals.

Group	Treatment					
	Days 1-7	Days 8-14	Days 15-21	Day 22-28	Day 29-35	Days 36-42
K0	Given only food and water	Given only food and water	Given only food and water	Given only food and water	Given only food and water	Given only food and water
K1	Given only food and water	Given food, water, and placebo aquadest (no extracts)	Given food, water, placebo aquadest (no extracts), and a combination of INH-RIF 500 mg/kg	Given food, water, placebo aquadest (no extracts), and a combination of INH-RIF 500 mg/kg	Given food, water, placebo aquadest (no extracts), and a combination of INH-RIF 500 mg/kg	Given food, water, placebo aquadest (no extracts), and a combination of INH-RIF 500 mg/kg
P1	Given only food and water	Given food, water and extract 200 mg/kg	Given food, water, extract 200 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 200 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 200 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 200 mg/kg and INH-RIF combination 500 mg/kg
P2	Given only food and water	Given food, water and extract 400 mg/kg	Given food, water, extract 400 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 400 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 400 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 400 mg/kg and INH-RIF combination 500 mg/kg
P3	Given only food and water	Given food, water and extract 600 mg/kg	Given food, water, extract 600 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 600 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 600 mg/kg and INH-RIF combination 500 mg/kg	Given food, water, extract 600 mg/kg and INH-RIF combination 500 mg/kg

Analysis of Total Phenol (mg/100 g) using the Spectrophotometric method (Follin Chiocalteau Phenol)
The analysis of total phenol (mg/100 g) was conducted using the spectrophotometric method described by Dóka *et al*.[38] Approximately 0.01 g of concentrated extract was incorporated into 5.0 mL of 85% methanol in a measuring flask. After pipetting 0.4 mL of the filtrate into a test tube, 0.4 mL of the Folin-Ciocalteau reagent was added. After vortexing for 6 minutes to ensure homogeneity, 4.2 mL of a 5% $NaCO_3$ solution was added, and the mixture was left at room temperature for half an hour. The absorbance of the medium was measured spectrophotometrically at 760 nm. The total phenol content was determined using the regression equation of the gallic acid standard curve, expressed as y = zx + b. The gallic acid equivalent (GAE) of the calculated data was expressed in milligrams per 100 grams.

Total flavonoid assay
Total flavonoid was assayed using the method described by Pai *et al*.[39]. Five millilitres of 100% methanol were combined with 0.01 g of a concentrated extract. 0.5 mL of filtrate was added with 1.0 ml of 2% $AlCl_3 \cdot 6H_2O$ reagent and 0.5 mL of 100% ethanol in a reaction tube. The mixture was vortexed to achieve homogeneity and subsequently allowed to equilibrate for half an hour at room temperature before the absorbance was measured at a wavelength of 415 nm. Quercetin was solubilised in a 50% ethanol distilled water solution at concentrations ranging from 0 to 30 mg to establish a standard curve. Flavonoids were quantified using the regression equation formula y = ax + b, expressed as mg per 100 g quercetin equivalent.

Antioxidant Capacity Analysis (mg/L GAEAC)
Antioxidant capacity analysis used a DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay.[40] 5.00 mL of 100% methanol was combined in a measuring flask with 0.01 g of concentrated ethanol extract. 0.25 mL of the filtrate and 0.25 mL of methanol were subsequently added, followed by the addition of 3.5 mL of 0.1 mM DPPH and vortexed. Then, 0.5 mL of the standard was introduced. The samples were incubated at 25°C for half an hour, and then the absorbance was measured at 517 nm. The antioxidant capacity was determined using the linear regression equation y = ax + b derived from the Gallic Acid standard curve. The gallic acid equivalent antioxidant capacity (GAEAC) was measured in milligrams per litre.

IC_{50} Antioxidant Activity Analysis (ppm)
The Inhibitory Concentration (IC_{50}) antioxidant activity was determined using a DPPH assay.[41] A concentrated extract (0.01 g) was dissolved in 100% methanol to reach a 5.00 mL final volume in a measuring flask. 1 mL of the filtrate was used to prepare multiple concentrations, and 0.1 mM DPPH solution was incorporated and the absorbance was measured at 517 nm in wavelength.

Antioxidant activity (Table 2) was measured by determining the 50% inhibition value (IC_{50}) using the formula as described by Marianne *et al*., [30], as shown below:

$$IC_{50} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} x100\%$$

The correlation between the IC value (%) and sample concentration (ppm) was computed from a linear regression equation of the form y = ax + b. Where x represents the concentration (ppm) at which the sample achieves a 50% reduction of 0.1 mM DPPH radicals.

8-OHdG Assay
The liver tissue was ultrasonicated to homogenise the tissue. The resulting mixture was centrifuged to separate the tissue from the supernatant. The supernatants formed were divided into micro tubes for analysis using the ELISA method. Every sample, reagent, and standard solution was made and kept at room temperature. The protocol for utilising the strips required placement within the frames, with unused strips stored at temperatures between 2°C and 8°C. The microplate well was with 50 µL of the standard, making certain that the standard solution's biotinylated antibody was not added to this well. For the sample wells, 40 µL of the samples were to be added, and in the wells standard (excluding the blank control wells), 10 µL of the anti-8-OHdG antibody was included along with 50 µL of streptavidin-H. The mixture was thoroughly stirred before the application of a sealant to the plate. The plate was incubated for an hour at 37°C. Following the removal of the sealant, the plate was washed five times with wash buffer, ensuring thorough soaking in 300 µL of wash buffer for thirty to sixty seconds for each wash. Using an automatic washing system, the medium was washed thoroughly before performing five washes with the wash buffer. Following washing, paper towels or other absorbent materials were used to blot the plate. Each well was then received 50 µL of substrate solution A and 50 µL of substrate solution B. After that, the plate was incubated for 10 minutes at 37°C in the dark with a new sealer. Then 50 µL of Stop Solution was applied to each well after the incubation period, with instant change in the colour instantly from blue to yellow. After ten minutes of adding the Stop Solution, each well's optical density (OD) value was determined using a microplate reader set at 450 nm to quickly determine.

Table 2: The Inhibitory Concentration (IC_{50}) category of antioxidant activity strength in vitro against DPPH

Value (ppm)	Intensity of IC_{50}
<50	Very active
50-100	Active
101-250	Medium
250-500	Weak
>500	Inactive

Results and Discussion

The analysis indicates that the 70% ethanol extract of *Spondias pinnata* leaves demonstrates significant antioxidant activity, elevated antioxidant capacity, and high levels of total phenols and flavonoids. The findings related to DNA oxidative stress markers, particularly 8-hydroxy-2'-deoxyguanosine (8-OHdG), indicated that levels of 8-OHdG were elevated in K1 compared to K0. These findings imply that oxidative stress is caused by isoniazid-rifampicin induction. According to the examination's findings, 8-OHdG levels were lower in P1, P2, and P3 than in K1. According to these findings, 70% ethanol extract of *Spondias pinnata* leaves possesses antioxidant properties that can reduce oxidative stress on DNA. The reduction in 8-OHdG levels was more pronounced at higher doses. These results prove that different doses of extracts can produce different levels of oxidative stress reduction. The results indicate a positive correlation between dosage and antioxidant power. The pathological basis of many diseases involves oxidative stress.[42] Liver injury from anti-TB drugs is associated with oxidative stress on mitochondria that causes liver cell injury, cell death, cholestasis, and accumulation of liver lipids.[43] When reactive oxygen species (ROS) interact with DNA molecules they produce 8-hydroxylamine (8-OHGua) or its nucleoside partner, deoxyguanosine (8-hydroxy-2'-deoxyguanosine). When hydroxyl radicals or singlet oxygen hydroxylate deoxyguanosine residues in DNA at the C8 site, 8-OHdG is created. Guanine is converted to thymine when there is a reading error. The rate of DNA release and strand breakage in liver tissue is likewise connected with 8-OHdG. 8-OHdG serves as a critical marker for assessing endogenous oxidative DNA damage.[44,45] The expression of 8-OHdG is prevalent in various forms of chronic liver disease.[32]
Antituberculosis medications can cause hepatotoxicity, which can lead to serious illness and even death. N-acetyltransferase 2 (NAT-2) converts isoniazid into acetylhydrazine and diacetylhydrazine. Acetylhydrazine isoniazid can hydrolyze, producing hepatotoxic hydrazine, whereas diacetylhydrazine is non-toxic. The cytochrome P450 (CYP) 2E1 isoenzyme can further metabolize isoniazid hydrazine into a reactive derivative or metabolite that is very toxic. Isoniazid interactions with electron transport chains, lipid peroxidation, alterations in the potential of the mitochondrial membrane, and

cytochrome c extrusion can disrupt normal cellular functions. Although rifampicin has a modest risk of hepatotoxicity, it can cause severe hepatotoxicity when used with isoniazid.[46] A higher risk of hepatotoxicity during hydrazine treatment for tuberculosis (TB) is linked to elevated redox imbalance, mitochondrial damage, and oxidative DNA damage. Hydrazine, a metabolite of isoniazid, has been identified as a significant factor contributing to isoniazid hepatotoxicity due to its interference with the mitochondrial electron transport chain. Beyond hepatotoxicity, oxidative DNA damage linked to isoniazid medication may cause other adverse effects. Polymerase-1 poly (ADP-ribose) (PARP-1) engages in DNA repair processes via a variety of signals and pathways of DNA damage that trigger its nuclear activity. Prolonged PARP-1 activation hinders mitochondrial ATP synthesis and cellular death by competing for intracellular NAD+. The mitochondrial thioredoxin system is directly suppressed, which is crucial for maintaining the balance between oxidation and reduction, this is one of the ways the mechanistic link between cellular signals for oxidative DNA damage and redox imbalance and physiological exposure to these substances. Hydrazine treatment in cell models significantly increases 8-OHdG. A study of tuberculosis patients undergoing isoniazid treatment found a correlation between serum isoniazid concentrations and urinary levels of 8-OHdG, which serves as a marker for oxidative DNA damage. 8-OHdG can be used as an early marker of clinical hepatotoxicity when isoniazid is administered.[47]

The primary source of plant extracts' biological potential is secondary metabolites. The most potent substances that combat inflammation and free radicals are polyphenols, which include flavonoids, phenolic, and organic acids.[48] According to Laksemi *et al*.[49], *Spondias pinnata* leaf ethanol extract contains terpenoids, polyphenols, and flavonoids. This study demonstrated the high phenolic and flavonoid content, strong antioxidant activity, and high antioxidant capacity of a 70% ethanol extract of *Spondias pinnata* leaves (Table 2). The study also showed a correlation between the secondary metabolite levels and antioxidant power of *Spondias pinnata* leaf extract and the IC_{50} (Table 3). Lower 8-OHdG levels in the P1, P2, and P3 groups relative to K1 in this study indicated that administering 70% ethanol extract of *Spondias pinnata* leaves could lower 8-OHdG levels in rats induced with isoniazid-rifampicin (Table 4 and Table 5).

Table 3: Secondary Metabolite Levels and Antioxidant Power of *Spondias pinnata* leaf extract expressed in mg/L Gallic Acid Equivalent Antioxidant Capacity (GAEAC) units and Inhibitory Concentration (IC_{50}).

Phenol (mg/100 g)	Flavonoid (mg/100 g)	Antioxidant capacity (mg/L GAEAC units)	IC_{50} (ppm)
2,710.17	6,888.41	11,919.48 mg/L	75.17

Table 4: Effect of Ethanol Extract of 70% *Spondias pinnata* cleared Liver 8-OHdG Levels

Group/treatment	8-OHdG (ng/mL) Mean ± SD	p
K0	279.56 ± 10.69	.000*
K1	480.32 ± 8.89	
P1	349.99 ± 6.05	
P2	327.38 ± 5.36	
P3	294.25 ± 1.87	

Notes:

K0=normal control/without isoniazid-rifampicin combination (0 mg/kg/day BW); K1=negative control treated with placebo aquadest (days 8 to 14) and isoniazid-rifampicin combination (500 mg/kg/day BW) for 28 days (days 15 to 42);

P1, P2, and P3= Groups treated with 70% *Spondias pinnata* leaf ethanol extract (200, 400, and 600 mg/kg/day BW) on days 8 to 42 and isoniazid-rifampicin combination (500 mg/kg/day BW) on days 15 to 42, respectively.

*P value showing a statistical significance based on the One-Way ANOVA test

According to reports, flavonoids reduce liver fibrosis, liver damage, and liver toxicity.[50] When it comes to treating cytotoxicity and hepatotoxicity, flavonoids are important.[51] One possible use for flavonoids is as antioxidants. Flavonoids' anti-oxidant qualities are facilitated by their functional hydroxyl groups, which effectively scavenge free radicals. Furthermore, flavonoids block the enzymes that produce reactive oxygen species and activate the glutathione peroxidase system as a protective enzyme. Polyphenols are reducing agents with antioxidant qualities that stabilize or delocalize unpaired electrons by donating electrons or hydrogen.[30] Phenols and flavonoids from natural sources exhibit individual iron-chelating properties, serving as effective antioxidants for the treatment of liver damage.[36] To protect against oxidative stress, which can result in oxidative DNA damage, plants primarily contain flavonoids and phenolics, which have antioxidant activity in liver cells. It is achieved by eliminating free radicals and preventing aggressive metabolites, identified by a decrease in 8-OHdG levels.[32,45] Flavonoids' aromatic system of mobile hydrogens undergoes polyhydroxylation, which gives them a strong ability to scavenge free radicals. The key structural components that enhance the flavonoids' anti-inflammatory and antioxidant qualities include a 4-carbonyl group and the presence of 3',4'-O-, and 5-hydroxyl substitutions on the B and A rings. This structural component strengthens defences against oxidative stress and damage by giving the radicals hydrogen or electrons. By doing this, they prevent the production and expression of inflammatory cytokines and stabilize cell membranes.[48] Because flavonoids and phenolics have a better antioxidant potential at higher dosages, this study demonstrated that higher doses of *Spondias pinnata* leaf extract administered to isoniazid-rifampicin-induced rats resulted in a more substantial drop in 8-OHdG levels.

This study's results align with several studies by Hazra *et al*.[36]. Hazra *et al*, assess the impact of enhancing 70% *Spondias pinnata* (SPME) methanol extract on hepatotoxicity brought on by too much iron. Biochemical and histological investigations support the conclusion that SPME guards against excessive iron-induced liver damage. Chaudhuri *et al*.[37] examine the components of methyl gallate (MG) and gallic acid (GA) that were separated from *Spondias pinnata* for their ability to treat hepatocellular damage and liver fibrosis due to iron excess.

Table 5: Difference in the effect of 70% ethanol extract of S*pondias pinnata)* cleared hepatic 8-OHdG levels between treatments

Treatment(I)	Treatment (J)	Mean difference (I-J)	Sig.	95% Confidence Interval	
				Lower bound	Upper bound
K0	K1	-200.753*	.000	-208.66	-192.85
	P1	-70.436*	.000	-78.34	-62.53
	P2	-47.817*	.000	-55.72	-39.91
	P3	-14.682*	.001	-22.59	-6.78
K1	K0	200.753*	.000	192.85	208.66
	P1	130.316*	.000	122.41	138.22
	P2	152.935*	.000	145.03	160.84
	P3	186.070*	.000	178.16	193.98
P1	K0	70.436*	.000	62.53	78.34
	K1	-130.316*	.000	-138.22	-122.41
	P2	22.619*	.000	14.71	30.53
	P3	55.754*	.000	47.85	63.66
P2	K0	47.817*	.000	39.91	55.72
	K1	-152.935*	.000	-160.84	-145.03
	P1	-22.619*	.000	-30.53	-14.71
	P3	33.135*	.000	25.23	41.04
P3	K0	14.682*	.001	6.78	22.59
	K1	-186.070*	.000	-193.98	-178.16
	P1	-55.754*	.000	-63.66	-47.85
	P2	-33.135*	.000	-41.04	-25.23

Note:

K0=normal control/without isoniazid-rifampicin combination (0 mg/kg/day BW); K1=negative control treated with placebo aquadest (days 8 to 14) and isoniazid-rifampicin combination (500 mg/kg/day BW) for 28 days (days 15 to 42);

P1, P2, and P3= Groups treated with 70% *Spondias pinnata* leaf ethanol extract (200, 400, and 600 mg/kg/day BW) on days 8 to 42 and isoniazid-rifampicin combination (500 mg/kg/day BW) on days 15 to 42, respectively.

*P value showing a statistical significance based on the Post Hoc test

Several biochemical tests and histological analyses were employed to evaluate the *in vitro* properties of iron chelation and the potential for reducing liver-induced excess iron toxicity from GA and MG *in vivo*. The *in vitro* characteristics of iron chelation and the possibility of improving liver-induced excess iron toxicity from GA and MG *in vivo* were evaluated by various biochemical tests and histological investigations. Because GA and MG have potent antioxidant and iron chelation properties, our investigation supports their ability to treat excessive liver damage caused by iron. Chaudhuri *et al*.[52] assess the possibility that glycosidic fractions of *Spondious pinnata* bark (SPW1) can alleviate iron overload hepatotoxicity. The fraction demonstrated excellent *in vitro* antioxidants and effectively scavenged free radicals associated with reactive oxygen and nitrogen species. Serum enzymes, ferritin, lipid peroxidation, liver fibrosis, antioxidant enzymes, and liver iron levels were all markedly restored when SPW1 was administered.

The aforementioned studies demonstrate that the bark of *spondias pinnata* can function as a hepatoprotective agent. A related study showed that *Plectranthus scutellarioides* leaf extract could mitigate the hepatotoxicity of Wistar rats treated with 50 mg/kg of isoniazid-rifampicin for 28 days. Chemical substances such as flavonoids, phenols, saponins, and triterpenoids are responsible for the protective impact.[53] *Phyllantus niruri* and *Ficus thonningii* (Blume) leaf extracts can reduce oxidative stress and hepatotoxicity induced by carbon tetrachloride in rats.[54,55] This shows herbal plants have great potential as hepatoprotective agents. Therefore, further study is needed to determine whether herbal plants might help people overcome the hepatotoxicity of chemicals and drugs.

Conclusion

The findings indicate that *Spondias pinnata* leaf extract may reduce 8-OHdG levels in rats administered isoniazid-rifampicin at dosages of 200 mg/kg bw, 400 mg/kg bw, and 600 mg/kg bw. The active constituents in *Spondias pinnata* exhibit antioxidant properties that safeguard rat liver DNA from oxidative stress induced by isoniazid-rifampicin. This study's findings indicate that leaf extract from *Spondias pinnata* may function as a hepatoprotective agent and may serve as a basis for more human research.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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