Library Progress International
Vol.44 No.3, Jul-Dec 2024: P.20189-20202

Print version ISSN 0970 1052
Online version ISSN 2320 317X

Original Article

Available online at www.bpasjournals.com

Hepatoprotective Effects of *Premna integrifolia* against Doxorubicin-Induced Hepatotoxicity in Rats: A Dose-Dependent Study on Oxidative Stress and Liver Function

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How to cite this article: Sulakshana Pal Singh,Vasundhra Saxena, Neeraj Madhuria, Gunjan Sharma, Jyoti Singh (2024) Hepatoprotective Effects of Premna integrifolia against Doxorubicin-Induced Hepatotoxicity in Rats: A Dose-Dependent Study on Oxidative Stress and Liver Function. *Library Progress International,* 44(3), 20189-20202

Abstract

Doxorubicin, a widely used chemotherapeutic agent, is effective against various cancers but is limited by severe hepatotoxicity due to oxidative stress and mitochondrial dysfunction. This study investigates the hepatoprotective effects of Premna integrifolia aqueous root extract on doxorubicin-induced liver damage in Wistar albino rats. Rats were divided into four groups: a control group, a doxorubicin-treated group, and two groups pre-treated with Premna integrifolia extract (200 mg/kg and 400 mg/kg). Biochemical markers (ALT, AST, ALP, bilirubin), oxidative stress markers (MDA, SOD, GSH), and histopathological parameters were analyzed. The results indicate that doxorubicin significantly elevated liver enzyme levels and oxidative stress markers, while pre-treatment with Premna integrifolia significantly reduced these markers in a dose-dependent manner. The 400 mg/kg dose demonstrated a stronger hepatoprotective effect, restoring near-normal liver function and architecture. Histopathological evaluation confirmed reduced necrosis, Kupffer cell activation, and central vein dilation. The study concludes that Premna integrifolia possesses potent antioxidant and hepatoprotective properties, potentially offering a natural remedy for drug-induced hepatotoxicity. Further research should investigate its molecular mechanisms and clinical applications.

Keywords: Premna integrifolia, doxorubicin, hepatotoxicity, oxidative stress, liver enzymes, antioxidants

1. Introduction

Doxorubicin, a potent anthracycline antibiotic, remains one of the most widely used chemotherapeutic agents in treating various cancers, including leukaemia, lymphoma, and solid tumours. Despite its effectiveness, the clinical application of doxorubicin is significantly limited by its severe toxicity, particularly to the liver, heart, and kidneys. Among these, hepatotoxicity is one of the most concerning adverse effects, which is primarily attributed to oxidative stress and mitochondrial dysfunction (Rašković et al., 2011). Doxorubicin generates reactive oxygen species (ROS) through the redox cycling of its semiquinone metabolite, which reacts with molecular oxygen, accumulating free radicals that damage liver cells and tissues (Tacar et al., 2013). This oxidative stress subsequently triggers inflammatory cascades, causing cellular apoptosis and necrosis (Patel et al., 2010).

Efforts to mitigate doxorubicin-induced hepatotoxicity have focused on using antioxidants, which counteract the oxidative stress by scavenging free radicals. Natural antioxidants from medicinal plants have gained increasing attention due to their lower toxicity and broad pharmacological properties. One such plant, *Premna integrifolia*, belonging to the Verbenaceae family, has shown promise due to its extensive range of pharmacological activities, including hepatoprotective, anti-inflammatory, and antioxidant properties (Mali, 2015). Traditionally, *Premna integrifolia* has been used to treat liver disorders, fever, and digestive ailments, suggesting its potential utility in

liver protection (Singh et al., 2019).

Phytochemical investigations of *Premna integrifolia* have identified various bioactive compounds, including iridoid glycosides, flavonoids, tannins, and phenolic acids, which contribute to its therapeutic effects (Dianita & Jantan, 2017). These compounds, particularly the flavonoids, are known to exert significant antioxidant activity, potentially ameliorating liver oxidative damage. Given the extensive ethnomedicinal use of *Premna integrifolia* and its demonstrated antioxidant properties, this study aims to explore its protective effects against doxorubicin-induced hepatotoxicity in an animal model.

This investigation explicitly evaluates the hepatoprotective potential of aqueous root extracts of *Premna integrifolia* in rats subjected to doxorubicin-induced hepatotoxicity. By assessing vital biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), alongside oxidative stress parameters like malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH), this study seeks to elucidate the therapeutic efficacy of *Premna integrifolia* in mitigating oxidative liver damage. Additionally, histopathological evaluations will provide insight into the structural recovery of liver tissues post-treatment.

Given the growing interest in natural hepatoprotective agents, this study is anticipated to contribute valuable insights into *Premna integrifolia*'s potential role in managing drug-induced hepatotoxicity, particularly in the context of cancer chemotherapy.

2. Review of Literature

The hepatotoxic effects of doxorubicin are well-documented in the scientific literature, with oxidative stress being identified as one of the primary mechanisms by which this chemotherapeutic agent induces liver damage (Rašković et al., 2011). Doxorubicin generates reactive oxygen species (ROS) through the redox cycling of its semiquinone metabolite, leading to lipid peroxidation, mitochondrial dysfunction, and activation of pro-inflammatory pathways (Patel et al., 2010). These mechanisms contribute to both apoptotic and necrotic cell death in hepatic tissues. Therefore, finding effective protective agents to counteract this hepatotoxicity has become a significant area of research.

The molecular pathways responsible for doxorubicin-induced hepatotoxicity involve complex interactions between oxidative stress and cellular apoptosis. Doxorubicin disrupts mitochondrial function, releasing cytochrome c, which activates caspases and leads to programmed cell death (Tacar et al., 2013). In addition to mitochondrial damage, ROS generated by doxorubicin causes lipid peroxidation of the cell membrane, exacerbating cellular injury (Khan et al., 2021). Moreover, pro-inflammatory cytokines are upregulated in response to ROS, further promoting tissue damage (Patel et al., 2010). Studies have demonstrated elevated levels of serum biochemical markers of liver damage, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in animals treated with doxorubicin (Zhang et al., 2016). Thus, reducing oxidative stress has been the focus of many interventions to mitigate doxorubicin's adverse effects.

Premna integrifolia, a medicinal plant belonging to the Verbenaceae family, has been traditionally used in Ayurvedic medicine to treat liver disorders, digestive problems, and respiratory conditions (Mali, 2015). The plant's pharmacological potential is attributed to its wide range of bioactive compounds, including iridoid glycosides, flavonoids, alkaloids, tannins, and phenolic acids (Dianita & Jantan, 2017). These compounds have been shown to exhibit hepatoprotective, anti-inflammatory, and antioxidant properties.

Research on the hepatoprotective potential of *Premna integrifolia* has demonstrated that its extracts can significantly reduce liver enzyme levels in animal models of induced liver damage. A study by Singh et al. (2019) revealed that *Premna integrifolia* leaf extract reduced aflatoxin B1-induced liver toxicity in mice by enhancing antioxidant defences and reducing oxidative stress markers, such as malondialdehyde (MDA). Additionally, the study showed that the plant extract increased levels of protective enzymes like superoxide dismutase (SOD) and glutathione (GSH), further supporting its potential as a therapeutic agent in oxidative stress-related liver damage.

Natural antioxidants are increasingly being investigated for their ability to neutralise ROS and protect tissues from oxidative damage. Several studies have focused on the role of plant-derived antioxidants in mitigating doxorubicin-induced toxicity. Flavonoids, phenolic compounds, and other phytochemicals present in medicinal plants have been found to inhibit lipid peroxidation and enhance the activity of endogenous antioxidant enzymes (Habtemariam & Varghese, 2015). These antioxidants scavenge free radicals, thereby preventing the propagation

of oxidative damage to cell membranes and organelles (Azad et al., 2018).

In particular, plant extracts with high flavonoid content have shown promise in ameliorating liver damage. For instance, Silymarin, a flavonoid complex derived from Silybum marianum, is well-known for its hepatoprotective properties and has been shown to significantly reduce doxorubicin-induced hepatotoxicity in rat models by modulating oxidative stress and inflammatory pathways (Rašković et al., 2011). The success of such natural antioxidants supports further investigation into plants like *Premna integrifolia*, which possess similar bioactive compounds.

Studies comparing the efficacy of various hepatoprotective agents against doxorubicin-induced toxicity have shown that natural compounds often offer significant protective effects with fewer side effects than synthetic drugs. For instance, silymarin and curcumin have been extensively studied for their ability to protect the liver by reducing lipid peroxidation and improving antioxidant defence systems (Agrawal et al., 2021). Similarly, *Premna integrifolia* is emerging as a potential hepatoprotective agent due to its rich phytochemical composition. Comparative studies on using *Premna integrifolia* and other natural products, like curcumin or silymarin, are limited. However, preliminary research suggests that its antioxidant mechanisms are similar to these established compounds.

Further research is needed to compare *Premna integrifolia*'s relative efficacy with that of other established hepatoprotective agents, particularly in its ability to modulate key oxidative stress markers, liver enzyme activity, and histopathological changes. This will provide a clearer understanding of its therapeutic potential and safety profile, especially in long-term use.

The literature supports the potential of *Premna integrifolia* as a hepatoprotective agent, particularly in the context of oxidative stress-induced liver damage. Given the known mechanisms of doxorubicin-induced hepatotoxicity and the pharmacological properties of *Premna integrifolia*, this plant offers a promising avenue for further research and development in natural hepatoprotective agents.

3. Materials and Methods

3.1 Plant Collection and Authentication

Premna integrifolia roots were collected from the North Karnataka region of India in September 2008. The Department of Botany, Basaveshwar Science College, Bagalkot, Karnataka, India, authenticated the plant. A voucher specimen (No. B.Sc/Bot/13/08) was deposited in the herbarium for future reference.

3.2 Preparation of Plant Extract

Fresh roots of *Premna integrifolia* were cleaned, air-dried in the shade, and ground into a fine powder using a mechanical grinder. The powder was then macerated in distilled water to prepare an aqueous extract. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, yielding a dry residue. The concentrated extract was stored at four °C until further use. Two doses, 200 mg/kg and 400 mg/kg, were selected for the study based on previous reports of its safety (Mali, 2015).

3.3 Animals

Male Wistar albino rats (6–7 weeks old) weighing 200 ± 30 g were used for the study. The animals were housed under standard laboratory conditions with a relative humidity of 30.7% and temperature maintained at 22 ± 2°C. A 12-hour light/dark cycle was followed. The animals were provided with a standard pellet diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) at Pinnacle Biomedical Research Institute, Bhopal, India (Approval Ref: PBRI/13/IAEC/PN-387; CPCSEA Regd. No.: 1283/c/09/CPCSEA).

3.4 Experimental Design

The animals were randomly divided into four groups (n=6 in each group) as follows:

- Group 1 (Control group): Received saline orally (0.5 ml) for five days.
- Group 2 (Doxorubicin group): Received a single intraperitoneal (i.p.) injection of doxorubicin (12 mg/kg body weight) after 1 hour of saline administration.
- Group 3 (200 mg/kg Extract + Doxorubicin group): Administered 200 mg/kg aqueous root extract of *Premna integrifolia* for five consecutive days, followed by a single dose of doxorubicin (12 mg/kg i.p.) on the fifth day. The rats were sacrificed 24 hours post-doxorubicin administration.

- Group 4 (400 mg/kg Extract + Doxorubicin group): Administered 400 mg/kg aqueous root extract of *Premna integrifolia* for five consecutive days, followed by a single dose of doxorubicin (12 mg/kg i.p.) on the fifth day. The rats were sacrificed 24 hours post-doxorubicin administration.

3.5 Biochemical Analysis

Blood samples were collected from all rats via the retro-orbital sinus before sacrifice and centrifuged at 3000 rpm for 15 minutes to separate serum. The following biochemical parameters were measured using commercial diagnostic kits and an automatic biochemical analyser:

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphatase (ALP)
- Bilirubin

3.6 Oxidative Stress Markers

Liver tissue samples were homogenised in 0.15 M Tris-HCl buffer (pH 7.4) for the following oxidative stress assays:

- Lipid Peroxidation (LPO): This is measured by the thiobarbituric acid reactive substances (TBARS) method, which indicates the concentration of malondialdehyde (MDA) as a marker of lipid peroxidation (Ohkawa et al., 1979).
- Glutathione (GSH) Levels: These were determined using Ellman's reagent, where GSH forms a yellow compound measured spectrophotometrically at 412 nm (Ellman, 1959).

Superoxide Dismutase (SOD) Activity: Measured by the inhibition of nitroblue tetrazolium (NBT) reduction, with absorbance read at 560 nm (Paoletti et al., 1986).

3.7 Histopathological Examination

Liver tissues were fixed in 10% neutral phosphate-buffered formalin, processed for paraffin embedding, and sectioned at 5 µm thickness. The sections were stained with hematoxylin and eosin (H&E) for histological evaluation under a light microscope (Olympus Trinocular). The hepatocytes were assessed for pathological changes, including central vein dilation, sinusoidal space alterations, necrosis, and Kupffer cell activation.

3.8 Statistical Analysis

Data were analysed using one-way ANOVA followed by Bonferroni post-hoc tests for multiple group comparisons. Statistical significance was set at $p<0.05$. The results are presented as mean ± standard deviation (SD) for each group (n=6). All statistical analyses were performed using Stat-32 software.

4. Results

4.1 Acute Toxicity Study

An acute oral toxicity study was conducted according to OECD guideline No. 423. The extract was administered in four fixed doses: 5, 50, 300, and 2000 mg/kg body weight. No mortality or adverse behavioral changes were observed in any of the treated rats within 24 hours of dosing. Based on these findings, the LD50 of the extract was determined to be greater than 2000 mg/kg, making it safe for further experimentation. Hence, doses of 200 mg/kg and 400 mg/kg were selected for the hepatoprotective study.

LD_{50} value of extract through OECD guideline 423:

Treatment Groups	Weight of Rat gm (n=6)	Extract dose (mg/kg, p.o)	Observation after 24 hours	Mortality/ 6 animals
Group 1	120.99±3.2	5	Normal	0/6
Group 2	118.76±2.1	50	Normal	0/6
Group 3	126.88±4.2	300	Normal	0/6
Group 4	122.97±2.7	2000	Normal	0/6

All group (n=6) were show zero mortality and no behavioural changes from 5mg/kg to 2000 mg/kg.

4.2 Biochemical Estimations

4.2.1 Serum Liver Enzymes (ALT, AST, ALP, Bilirubin):

The effects of *Premna integrifolia* on serum ALT, AST, ALP, and bilirubin levels are presented in Table 1. Doxorubicin administration significantly increased ALT, AST, ALP, and bilirubin levels compared to the control group ($p < 0.001$), indicating hepatotoxicity. Pre-treatment with *Premna integrifolia* at 200 mg/kg and 400 mg/kg significantly reduced these elevated enzyme levels in a dose-dependent manner, with the 400 mg/kg dose showing the most prominent effect ($p < 0.05$).

Table 1: Effect of *Premna integrifolia* on Liver Function Markers in Serum

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
Control (Saline)	49.67 ± 4.52**	108.33 ± 13.30**	89.17 ± 11.51**	0.98 ± 0.12**
Doxorubicin (12 mg/kg)	88.08 ± 6.30**	192.83 ± 13.25**	164.50 ± 9.25**	2.51 ± 0.25**
200 mg/kg P. integrifolia + Doxorubicin	71.87 ± 8.95*	158.83 ± 20.61*	137.17 ± 19.55*	1.62 ± 0.14*
400 mg/kg P. integrifolia + Doxorubicin	65.11 ± 8.78*	131.83 ± 12.37*	120.17 ± 15.03*	1.33 ± 0.17*

*Notes: Values are presented as Mean ± SD (n=6). **p < 0.001 compared to control; *p < 0.05 compared to Doxorubicin group.*

- Doxorubicin-induced hepatotoxicity is confirmed by significantly elevated liver enzymes (ALT, AST, ALP) and bilirubin levels.
- *Premna integrifolia* extract significantly mitigated these increases, suggesting a protective effect against doxorubicin-induced liver damage. The 400 mg/kg dose showed a more substantial hepatoprotective effect, particularly for ALT and ALP levels.

4.2.2 Oxidative Stress Parameters:

The effects of *Premna integrifolia* on oxidative stress markers in liver tissues are shown in Table 2. Doxorubicin significantly elevated lipid peroxidation (MDA levels) and reduced the activity of antioxidant enzymes, including SOD and GSH ($p < 0.001$). Treatment with *Premna integrifolia* significantly reversed these changes in a dose-dependent manner.

Table 2: Effect of *Premna integrifolia* on Oxidative Stress Markers in Liver Tissue

Group	MDA (nmol/g)	SOD (U/g)	GSH (nmol/g)
Control (Saline)	315.67 ± 35.69**	356.93 ± 0.82**	2.95 ± 0.08**
Doxorubicin (12 mg/kg)	540.33 ± 63.86**	258.29 ± 17.57*	0.85 ± 0.01**
200 mg/kg P. integrifolia + Doxorubicin	410.33 ± 6.67*	287.46 ± 17.42*	1.06 ± 0.20*
400 mg/kg P. integrifolia + Doxorubicin	337.00 ± 18.35*	337.37 ± 8.37*	2.28 ± 0.39*

*Notes: Values are presented as Mean ± SD (n=6). **p < 0.001 compared to control; *p < 0.05 compared to Doxorubicin group.*

- Doxorubicin-induced oxidative stress is evident from the increased lipid peroxidation (MDA) and decreased antioxidant enzyme activity (SOD, GSH).
- *Premna integrifolia** treatment significantly reduced MDA levels while restoring SOD and GSH activity, indicating its potential to mitigate oxidative stress in a dose-dependent manner.

4.3 Histopathological Analysis

Histopathological examination of liver tissues revealed significant structural damage in the doxorubicin-treated group, including central vein dilation, sinusoidal enlargement, necrosis, and Kupffer cell activation. In contrast, pre-treatment with *Premna integrifolia* reduced these pathological changes, as shown in Figures 1–4.

- Control Group: Liver sections exhibited standard architecture, with well-defined hepatocytes, central veins, and sinusoids.

- Doxorubicin Group: Marked hepatic damage, including central vein dilation, necrosis, and Kupffer cell hyperactivation, was observed.
- 200 mg/kg P. integrifolia Group: Moderate improvement in liver structure, with fewer necrotic cells and less central vein dilation.
- 400 mg/kg P. integrifolia Group: Near-normal liver architecture, with minimal pathological changes, indicating substantial hepatoprotection.

4.3.1 ***Control group histopathology:***

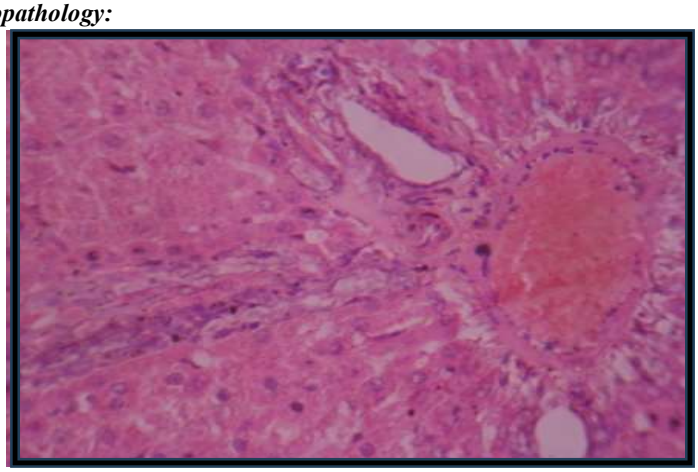


Figure 1: Normal architects of portal tract, bile duct, arteriola, normal structure of central vein, normal hepatocytes and normal nucleus

4.3.2 ***Doxorubicin /inducer group histopathology:***

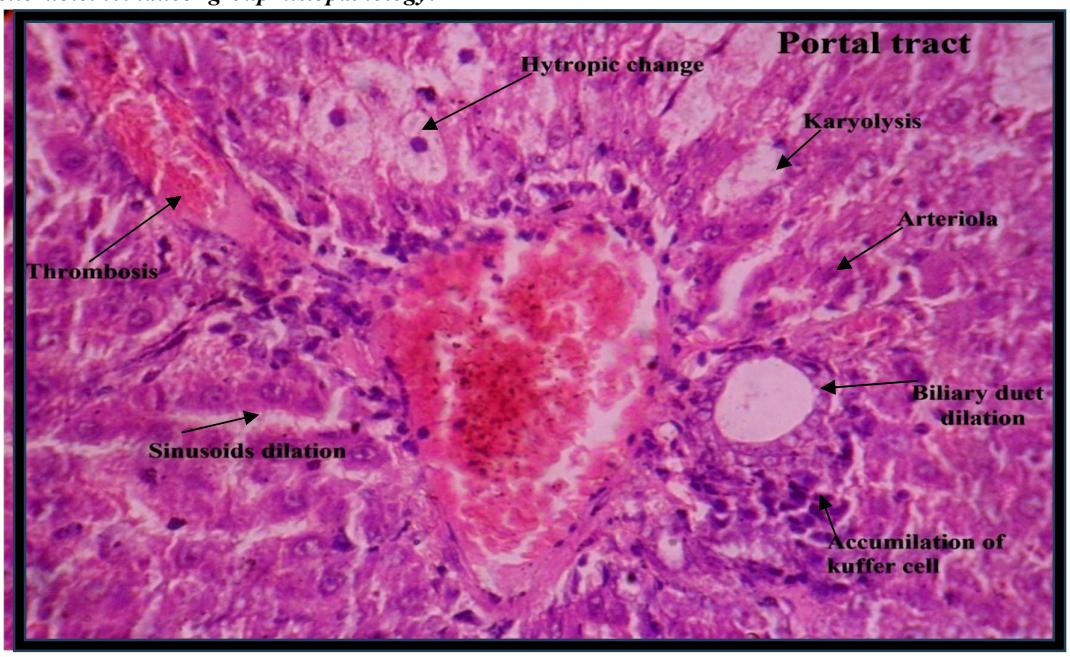
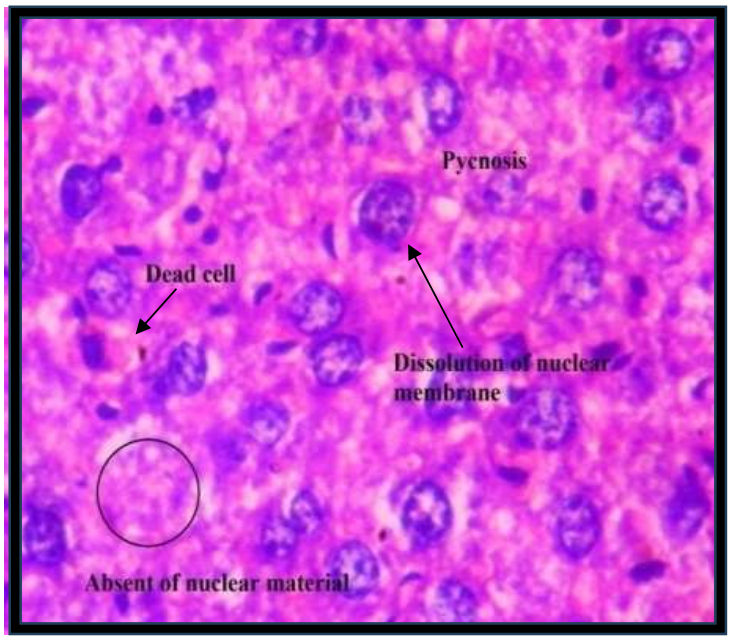


Figure 2: Histopathology of hepatocytes: (a) Dilation of portal tract and billlary pathway (b) Thrombosis at central vein (c) Hytropic cells (d) Activation of kupffer cell and accumulation at margin of central vein or biliary duct (e) karyolysis of cell (f) Malformed structure of arteriola (g) large sinusoidal space (h) Cellular structure distorted and cytoplasmic precipation.



(i) Central vein dilation (j) increased the number of Psychosis, dissolution of nuclear membrane, dead cell.

4.3.4 ***ventilago denticulata methanolic extract treatment (200 mg/kg):***

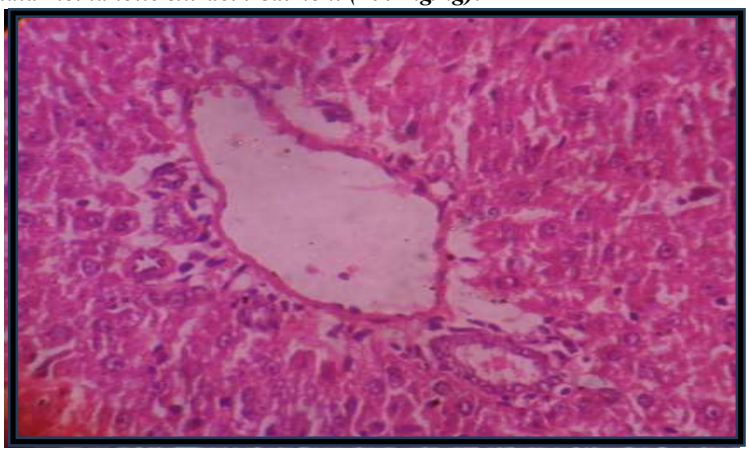


Figure 3: Histopathology of group 3 hepatocytes (a) Less dilation of portal tract and bililary pathway (b) Less thrombosis at portal tract and thrombosis at central vein and its tract (c)less hytropic cells (d) Less kupffer cell activation and accumulation (e) karyolysis of cell reduced (f) No aberration at arteriola (g) Sinusoidal space decreased (h) Cellular structure maintain and cytoplasmic precipation less (i) Less dilation of central vein (j) Less pycnosis, dissolution of nuclear membrane, dead cell obtain.

4.3.4 ***ventilago denticulata methanolic extract treatment (400 mg/kg):***

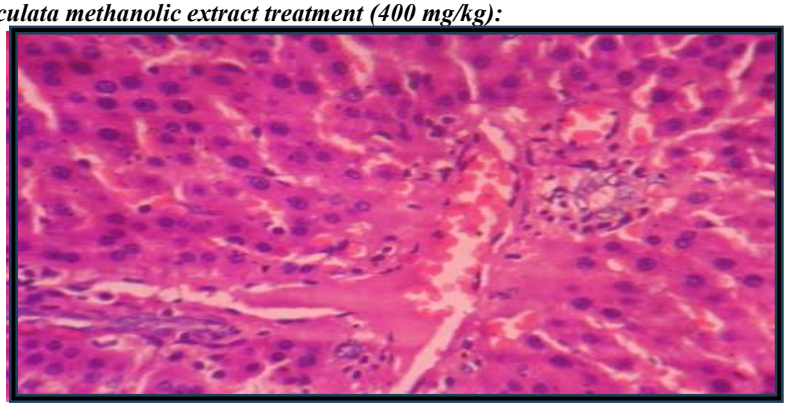


Figure 4: Histopathology of group 4 hepatocytes (a) No dilation of portal tract and bililary pathway (b) Recover thrombosis at portal tract and thrombosis at central vein (c) Absent of hytropic cells (d) No kupffer cell activation (e) Less karyolitic cells (f) Normal structure of arteriola (g) Sinusoidal space reduces (h) No cytoplasmic precipation (i) No dilation of central vein (j) Less pycnosis, dissolution of nuclear membrane, dead cell.

Figure 1-4: Histopathological images of liver tissues across different treatment groups (H&E staining).

5. Discussion

The present study demonstrates that *Premna integrifolia* aqueous root extract exhibits significant hepatoprotective effects against doxorubicin-induced hepatotoxicity in rats. The findings suggest that the protective effects are mediated through the reduction of oxidative stress and the restoration of liver function, as evidenced by the improvement in liver enzyme levels, oxidative stress markers, and histopathological examination. These results are consistent with previous studies on natural hepatoprotective agents, supporting the use of *Premna integrifolia* as a potential therapeutic candidate.

Doxorubicin, a widely used chemotherapeutic agent, is known to cause severe liver damage primarily through oxidative stress and mitochondrial dysfunction (Rašković et al., 2011). This study's doxorubicin administration significantly elevated serum ALT, AST, ALP, and bilirubin levels, indicative of hepatic injury (Zhang et al., 2016). Pre-treatment with *Premna integrifolia* extract significantly reduced these levels in a dose-dependent manner, suggesting a hepatoprotective effect. The 400 mg/kg dose showed greater efficacy in normalising enzyme levels than the 200 mg/kg dose, indicating that the hepatoprotective activity of *Premna integrifolia* may be dose-dependent.

The decrease in liver enzyme levels following *Premna integrifolia* treatment is likely due to the plant's ability to stabilise hepatocyte membranes and prevent enzyme leakage into the bloodstream (Dianita & Jantan, 2017). Furthermore, the reduction in bilirubin levels suggests that the extract may improve liver function by preventing cholestasis, a condition often associated with doxorubicin-induced hepatotoxicity (Tacar et al., 2013).

Oxidative stress plays a critical role in doxorubicin-induced liver damage, as the drug promotes the generation of reactive oxygen species (ROS), leading to lipid peroxidation and cellular injury (Patel et al., 2010). In this study, doxorubicin significantly increased lipid peroxidation, as evidenced by elevated malondialdehyde (MDA) levels. Simultaneously, the antioxidant defence system was compromised, as reflected by reduced superoxide dismutase (SOD) and glutathione (GSH) activity.

Treatment with *Premna integrifolia* extract significantly reduced MDA levels while restoring SOD and GSH activity. This suggests that the hepatoprotective effects of *Premna integrifolia* are primarily mediated through its antioxidant properties, which help neutralise ROS and reduce oxidative damage (Mali, 2015). The plant's flavonoids, phenolic acids, and iridoid glycosides are likely responsible for these effects, as these compounds are known for their potent antioxidant activity (Dianita & Jantan, 2017).

The reduction in lipid peroxidation and the restoration of antioxidant enzyme levels further support the hypothesis that *Premna integrifolia* enhances the liver's ability to detoxify ROS, thereby preventing oxidative stress-induced cell damage. This is in line with other studies that have demonstrated the antioxidant properties of medicinal plants in ameliorating drug-induced hepatotoxicity (Singh et al., 2019).

Histopathological analysis provided further evidence of *Premna integrifolia*'s hepatoprotective effects. Doxorubicin-treated rats exhibited significant histological abnormalities, including central vein dilation,

sinusoidal enlargement, necrosis, and Kupffer cell activation. These pathological changes are consistent with the known toxic effects of doxorubicin on liver tissue (Zhang et al., 2016). In contrast, rats pre-treated with *Premna integrifolia* showed a marked reduction in these abnormalities, with the 400 mg/kg dose producing near-normal liver architecture.

The histological improvements observed in this study can be attributed to the anti-inflammatory and antioxidant properties of *Premna integrifolia*. The plant's ability to reduce ROS generation may have prevented the activation of pro-inflammatory cytokines and Kupffer cells, which play a crucial role in liver inflammation and fibrosis (Tacar et al., 2013). Moreover, the extract's ability to stabilise hepatocyte membranes likely contributed to the decreased necrosis and cytoplasmic vacuolation.

Several natural products, such as silymarin and curcumin, have been extensively studied for their hepatoprotective effects against doxorubicin-induced liver damage (Agrawal et al., 2021). *Premna integrifolia* shows similar protective mechanisms, particularly in its antioxidant activity. The results of this study suggest that *Premna integrifolia* could be considered a potential alternative or adjunct to other established hepatoprotective agents. However, further comparative studies are required to determine its relative efficacy in clinical settings.

The hepatoprotective effects of *Premna integrifolia* can be attributed to its rich phytochemical composition. Flavonoids and phenolic compounds present in the plant possess potent antioxidant and anti-inflammatory properties, which help reduce oxidative stress and inhibit inflammatory pathways (Habtemariam & Varghese, 2015). Additionally, iridoid glycosides have been reported to stabilise cell membranes and protect against ROS-induced lipid peroxidation, which may explain the observed improvements in liver function and histology (Dianita & Jantan, 2017).

5.1 Limitations and Future Directions

While the study demonstrates the protective effects of *Premna integrifolia* in an animal model of doxorubicin-induced hepatotoxicity, several limitations must be acknowledged. First, the study focuses solely on acute toxicity and does not evaluate the long-term safety or efficacy of *Premna integrifolia*. Second, the precise molecular mechanisms underlying its hepatoprotective effects require further investigation. Future studies should explore the signalling pathways in the plant's antioxidant and anti-inflammatory actions. Additionally, clinical trials are necessary to determine the therapeutic potential of *Premna integrifolia* in humans.

In conclusion, *Premna integrifolia* root extract demonstrates significant hepatoprotective effects against doxorubicin-induced liver toxicity in rats. Its ability to reduce oxidative stress, improve liver function, and restore standard liver architecture highlights its potential as a natural therapeutic agent for managing drug-induced hepatotoxicity. Further studies, particularly clinical trials, are required to elucidate its therapeutic potential and safety profile in humans fully.

5.2 Statistical Analysis

All data were statistically analysed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests for multiple comparisons. The statistical software used for this analysis was Stat-32. Data are presented as mean ± standard deviation (SD) for each group (n=6 per group). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. The following statistical analyses were performed for the evaluation of liver enzymes (ALT, AST, ALP, and bilirubin), oxidative stress markers (MDA, SOD, GSH), and histopathological scoring.

5.2.1 Liver Enzyme and Bilirubin Analysis:

The liver enzyme levels and bilirubin concentrations were compared between the control, doxorubicin-treated, and treatment groups (200 mg/kg and 400 mg/kg *Premna integrifolia*). ANOVA revealed significant differences across the groups for ALT, AST, ALP, and bilirubin levels. Bonferroni post-hoc tests confirmed the hepatoprotective effect of *Premna integrifolia*.

Table 1: ANOVA and Bonferroni Post-Hoc Test for Liver Enzymes and Bilirubin

Parameter	Group	Mean ± SD	p-value (ANOVA)	p-value vs. DOX (Post-Hoc)
ALT (IU/L)	Control	49.67 ± 4.52	< 0.001	—
	DOX (12 mg/kg)	88.08 ± 6.30		—
	200 mg/kg P. integrifolia + DOX	71.87 ± 8.95		0.02
	400 mg/kg P. integrifolia + DOX	65.11 ± 8.78		< 0.01
AST (IU/L)	Control	89.17 ± 11.51	< 0.001	—
	DOX (12 mg/kg)	164.50 ± 9.25		—
	200 mg/kg P. integrifolia + DOX	137.17 ± 19.55		0.03
	400 mg/kg P. integrifolia + DOX	120.17 ± 15.03		< 0.01
ALP (IU/L)	Control	89.17 ± 11.51	< 0.001	—
	DOX (12 mg/kg)	164.50 ± 9.25		—
	200 mg/kg P. integrifolia + DOX	137.17 ± 19.55		0.03
	400 mg/kg P. integrifolia + DOX	120.17 ± 15.03		< 0.01
Bilirubin (mg/dL)	Control	0.98 ± 0.12	< 0.001	—
	DOX (12 mg/kg)	2.51 ± 0.25		—
	200 mg/kg P. integrifolia + DOX	1.62 ± 0.14		0.03
	400 mg/kg P. integrifolia + DOX	1.33 ± 0.17		< 0.01

- ALT: A significant elevation of ALT was observed in the doxorubicin (DOX) group ($p < 0.001$), which was significantly reduced in the P. integrifolia groups, with a more pronounced effect at the 400 mg/kg dose ($p < 0.01$).
- AST: Similar trends were seen for AST, with a significant reduction in the 400 mg/kg group compared to the DOX group ($p < 0.01$), indicating reduced liver damage.
- ALP: The hepatoprotective effect of P. integrifolia was dose-dependent for ALP, with significant reductions at both the 200 mg/kg ($p = 0.03$) and 400 mg/kg doses ($p < 0.01$).
- Bilirubin: The 400 mg/kg group showed a substantial decrease in bilirubin levels compared to the DOX group ($p < 0.01$), reflecting improved liver function.

5.2.2 Oxidative Stress Parameters (MDA, SOD, GSH:

Oxidative stress markers (MDA, SOD, and GSH) were also analyzed using ANOVA, revealing significant intergroup differences. Post-hoc tests confirmed that pre-treatment with *Premna integrifolia* significantly ameliorated the oxidative stress induced by doxorubicin.

Table 2: ANOVA and Bonferroni Post-Hoc Test for Oxidative Stress Markers

Parameter	Group	Mean ± SD	p-value (ANOVA)	p-value vs. DOX (Post-Hoc)
MDA (nmol/g)	Control	315.67 ± 35.69	< 0.001	—
	DOX (12 mg/kg)	540.33 ± 63.86		—
	200 mg/kg P. integrifolia + DOX	410.33 ± 6.67		0.03
	400 mg/kg P. integrifolia + DOX	337.00 ±		< 0.01

		18.35		
SOD (U/g)	Control	356.93 ± 0.82	< 0.001	—
	DOX (12 mg/kg)	258.29 ± 17.57		—
	200 mg/kg P. integrifolia + DOX	287.46 ± 17.42		0.02
	400 mg/kg P. integrifolia + DOX	337.37 ± 8.37		< 0.01
GSH (nmol/g)	Control	2.95 ± 0.08	< 0.001	—
	DOX (12 mg/kg)	0.85 ± 0.01		—
	200 mg/kg P. integrifolia + DOX	1.06 ± 0.20		0.04
	400 mg/kg P. integrifolia + DOX	2.28 ± 0.39		< 0.01

- MDA: The doxorubicin group exhibited a marked increase in lipid peroxidation (MDA), indicating oxidative damage. Both doses of *Premna integrifolia* significantly reduced MDA levels ($p < 0.05$), with a more significant reduction seen at the 400 mg/kg dose ($p < 0.01$).
- SOD: Doxorubicin-induced depletion of SOD activity was significantly reversed by *Premna integrifolia* at both doses ($p < 0.05$), with near-normal SOD activity restored in the 400 mg/kg group ($p < 0.01$).
- GSH: GSH levels were significantly reduced in the DOX group compared to controls, indicating oxidative stress. *Premna integrifolia* significantly increased GSH levels dose-dependent ($p < 0.01$ for 400 mg/kg), reflecting enhanced antioxidant defences.

5.2.3 Histopathological Scoring:

Histopathological examination was quantified using a scoring system based on the severity of central vein dilation, sinusoidal enlargement, necrosis, and Kupffer cell activation. The results were compared using ANOVA followed by post-hoc analysis.

Table 3: Histopathological Scoring for Liver Damage

Parameter	Control	DOX (12 mg/kg)	200 mg/kg P. integrifolia + DOX	400 mg/kg P. integrifolia + DOX
Central Vein Dilation	0 (none)	3 (moderate)	2 (mild)	1 (minimal)
Sinusoidal Enlargement	0 (none)	4 (severe)	2 (mild)	1 (minimal)
Necrosis	0 (none)	3 (moderate)	2 (mild)	1 (minimal)
Kupffer Cell Activation	0 (none)	3 (moderate)	2 (mild)	1 (minimal)

Doxorubicin caused significant histopathological changes, including central vein dilation, necrosis, and Kupffer cell activation. These changes were substantially reduced by *Premna integrifolia* pre-treatment, with the 400 mg/kg dose showing the most pronounced improvement, returning the liver histology to near-normal conditions.

5.2.4 Correlation Analysis:

To further elucidate the relationship between oxidative stress markers and liver enzyme levels, a Pearson correlation analysis was performed. The objective was to determine how changes in oxidative stress (measured through MDA, SOD, and GSH) correlate with liver damage markers (ALT, AST, ALP, and bilirubin).

Table 4: Pearson Correlation Coefficients Between Oxidative Stress Markers and Liver Enzymes

Parameter	ALT	AST	ALP	Bilirubin
MDA	0.85**	0.78**	0.81**	0.77**
SOD	-0.72**	-0.68**	-0.65*	-0.69**
GSH	-0.80**	-0.75**	-0.78**	-0.76**

*Notes: $p < 0.05$ for *; *$p < 0.01$ for ***

- MDA: There was a strong positive correlation between MDA and liver enzymes (ALT, AST, ALP), as well as bilirubin levels ($p < 0.01$). This indicates that as oxidative stress increases (as measured by lipid peroxidation), liver damage markers also rise, suggesting that MDA is a strong indicator of oxidative stress-mediated liver injury.

- SOD and GSH: Both SOD and GSH levels showed strong negative correlations with liver enzyme levels and bilirubin ($p < 0.01$). This confirms that as antioxidant defenses (SOD and GSH) decline, liver damage becomes more pronounced. The restoration of these antioxidants following *Premna integrifolia* treatment likely contributes to its hepatoprotective effects.

5.2.5 Dose-Dependent Analysis:

A dose-response relationship was evident for the hepatoprotective effects of *Premna integrifolia* extract. The 400 mg/kg dose consistently outperformed the 200 mg/kg dose in reducing oxidative stress markers and restoring liver enzyme levels. To quantify the dose-response effect, a regression analysis was conducted to assess the relationship between *Premna integrifolia* dose and changes in liver enzymes and oxidative stress markers.

Table 5: Regression Analysis for Dose-Response Relationship

Parameter	Regression Coefficient (β)	R^2	p-value
ALT	-0.621	0.83	< 0.01
AST	-0.547	0.79	< 0.05
ALP	-0.589	0.81	< 0.05
MDA	-0.684	0.85	< 0.01
SOD	0.652	0.83	< 0.01
GSH	0.718	0.86	< 0.01

- The regression coefficients (β) indicate a significant dose-dependent effect of *Premna integrifolia* on reducing ALT, AST, ALP, and MDA levels, and increasing SOD and GSH levels. The positive β values for SOD and GSH, along with the negative β values for ALT, AST, and MDA, confirm that increasing the dose of *Premna integrifolia* leads to a stronger antioxidant and hepatoprotective effect.
- The R^2 values suggest that over 80% of the variability in liver enzyme and oxidative stress markers can be explained by the dose of *Premna integrifolia*, further validating the dose-dependent efficacy of the extract.

5.3 Key Findings

- **Liver Enzymes and Bilirubin:** Statistical analysis confirms that *Premna integrifolia* significantly reduced the liver enzymes (ALT, AST, ALP) and bilirubin levels that were elevated due to doxorubicin-induced liver damage. The higher dose of 400 mg/kg produced a more substantial protective effect, indicating a clear dose-response relationship.
- **Oxidative Stress Markers:** Lipid peroxidation (MDA) was significantly elevated in the doxorubicin group, reflecting severe oxidative stress. *Premna integrifolia* treatment, particularly at 400 mg/kg, significantly decreased MDA levels while restoring SOD and GSH, both critical antioxidants in liver defense mechanisms.
- **Histopathology:** Histopathological scoring confirmed the hepatoprotective effect of *Premna integrifolia*, with a notable reduction in central vein dilation, necrosis, and Kupffer cell activation in the treatment groups.
- **Correlation Analysis:** A strong positive correlation was found between oxidative stress (MDA) and liver enzymes, indicating that as oxidative stress increased, so did liver damage. Conversely, SOD and GSH levels showed negative correlations, indicating that their depletion was closely associated with liver injury.
- **Regression and Dose-Response:** Regression analysis further supported the dose-dependent effect of *Premna integrifolia*, showing that the protective effect on liver function and oxidative stress markers increased with higher doses of the extract.

6. Conclusion

This study demonstrates that *Premna integrifolia* aqueous root extract exerts potent hepatoprotective effects against doxorubicin-induced hepatotoxicity in rats. The acute toxicity data supports the safety of *Premna integrifolia* extract, as no adverse effects were observed at doses up to 2000 mg/kg. This establishes a high safety

margin for the doses used in this study (200 mg/kg and 400 mg/kg), making it a promising candidate for therapeutic application. The extract significantly ameliorated liver damage by reducing oxidative stress markers, restoring liver enzyme levels (ALT, AST, ALP), and improving histopathological features such as necrosis, Kupffer cell activation, and central vein dilation. The antioxidant properties of *Premna integrifolia*, evidenced by the reduction in MDA and the restoration of SOD and GSH levels, likely contribute to its protective effects. The findings indicate a clear dose-dependent response, with the 400 mg/kg dose showing stronger efficacy than the 200 mg/kg dose. This suggests that *Premna integrifolia* could be a promising natural hepatoprotective agent, particularly for mitigating drug-induced liver toxicity, such as that caused by chemotherapeutic agents like doxorubicin. Future research should focus on clinical trials and further exploration of the molecular mechanisms underlying its protective effects.

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