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***Research Article** (Open Access)*

PROTECTIVE EFFECT OF AMLA ON ENDOSULFAN-INDUCED KIDNEY DAMAGE IN SWISS ALBINO MICE

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ABSTRACT
The widespread use of organochemicals in agriculture has caused significant health and environmental concerns. Among these, Endosulfan, an organochlorine pesticide, is particularly hazardous due to its persistence and association with cancer, endocrine disruption, immune dysfunction, and reproductive toxicity. Despite being banned globally under the Stockholm Convention in 2011, its continued use in some countries remains a major challenge.
This study investigates the nephrotoxic effects of Endosulfan and evaluates the potential protective role of *Emblica officinalis* (Amla), a known antioxidant, in mitigating kidney damage. Swiss albino mice were orally administered Endosulfan at its maximum tolerated dose daily for four weeks. This was followed by treatment with Amla powder dissolved in drinking water at its maximum tolerated dose for an additional four weeks. Histopathological examination of kidney tissues was conducted at intervals using light microscopy to assess structural changes. The findings revealed significant histological damage in the renal cortex and tubules, including necrosis and glomerular clumping, following Endosulfan exposure. Treatment with Amla demonstrated ameliorative effects, reducing necrotic lesions and improving the histological integrity of renal tissues. These results highlight the potential of Amla as a natural nephroprotective agent against Endosulfan-induced toxicity.

Keywords: *Endosulfan, Swiss Albino Mice, Amla Powder, Nephrotoxicity, Antioxidants*

INTRODUCTION
Agricultural pests are a major challenge, responsible for nearly one-third of global crop production losses annually. This issue is further compounded by the emergence of new pests and diseases, necessitating the widespread use of pesticides. While pesticides are effective in controlling pests, their indiscriminate use often impacts non-target organisms, including humans, posing significant health risks. Among these chemicals, Endosulfan, a broad-spectrum organochlorine insecticide, has gained attention due to its persistence in the environment and severe toxic effects. It is commonly used on crops like cereals, vegetables, coffee, and potatoes, and though it tends to remain localized to its application area, high concentrations have been reported in various regions, underscoring its extensive usage.
Endosulfan has been linked to multiple adverse effects, including endocrine disruption, immune suppression, reproductive toxicity, and genotoxicity (Pandey *et al.,* 1990). Studies have also reported its potential to induce kidney diseases of unknown etiology (Ghosh *et al.,*), with nephrotoxicity being one of its significant manifestations. Singh and Pandey (1980) highlighted its impact on the kidneys of Swiss albino mice, demonstrating its influence on drug-metabolizing enzymes. Due to its hazardous effects on human health and ecosystems, the Stockholm Convention in 2011 recommended a global ban on Endosulfan. However, its usage persists in several countries, exacerbating public health concerns (Matthew, 2011).
Amla (*Emblica officinalis*), commonly known as Indian gooseberry, is a well-documented medicinal plant renowned for its rich antioxidant and anti-inflammatory properties. Its bioactive constituents, including vitamin C, flavonoids, and tannins, contribute to its protective effects against oxidative stress-induced

cellular damage. Previous research has demonstrated the ameliorative potential of Amla extract in nephrotoxicity caused by various toxicants such as dimethoate (Rao et al , 2024). Furthermore, Naik *et al.,* (2005) demonstrated Amla's ability to enhance enzymatic defense mechanisms and reduce oxidative stress. Malik *et al.,* (2016) and Purena *et al.,* (2018) have reported the protective role of Amla in mitigating cisplatin-induced nephrotoxicity. Additionally, Sinha *et al.,* (2021) showed that Amla fruit extract exhibited nephroprotective effects against malachite green toxicity in fish. These studies collectively suggest that Amla possesses significant nephroprotective potential across different toxicological models.

However, despite the extensive research on Amla's antioxidant and nephroprotective properties, no prior study has investigated its role in countering endosulfan-induced nephrotoxicity in mammals. Given the widespread use of endosulfan and its well-documented nephrotoxic effects (Khan, 2014), exploring potential natural therapeutic agents to mitigate its toxicity is of critical importance. The present study, for the first time, establishes the nephroprotective effect of Amla extract against endosulfan-induced nephrotoxicity in a mammalian model. By evaluating histopathological alterations in kidney tissues, this study aims to provide novel insights into the protective role of Amla against endosulfan toxicity.

The findings of this study not only expand the existing knowledge on Amla's nephroprotective effects but also contribute to the broader field of environmental toxicology and herbal therapeutics. By demonstrating the potential of Amla as a natural intervention for pesticide-induced nephrotoxicity, this research underscores its relevance in developing alternative strategies to combat renal damage caused by environmental toxicants.

MATERIALS AND METHODS

***Experimental Animals*:** The study was conducted using healthy male *Mus musculus* (Swiss albino mice), each weighing approximately 28 ± 2 grams. The animals were procured from the Molecular Cell Biology Laboratory, Department of Zoology, Patna University. Standard laboratory conditions were maintained throughout the experiment. The mice were housed in cages with rice husk bedding, provided with curved nozzles and bottles for water supply. A 12-hour light-dark cycle was maintained at a constant temperature of 22 ± 2°C. Food and water were provided *ad libitum*, with a nutritionally balanced, mixed-formulated feed prepared by the laboratory.

***Chemicals*:** Endosulfan (35% emulsifiable concentrate, EC) was used as the pesticide in this study. It was procured from Excel India Private Limited, Mumbai.

***Plant Materials*:** The fresh dried fruit of *Emblica officinalis* (Amla) was purchased from a local herbal store in Patna. The dried fruit was ground into a fine powder using a mortar and pestle. For experimental use, the powder was dissolved in distilled water to prepare an aqueous extract. The extract was freshly prepared before each administration to ensure its efficacy.

Methodology

Phase 1: Determination of Maximum Tolerated Dose (MTD) of Endosulfan

To establish the MTD of Endosulfan, mice were divided into groups of six and housed in separate cages. Each group received a single daily dose of Endosulfan in drinking water at concentrations of 0.6 ppm, 0.24 ppm, 0.48 ppm, 0.96 ppm, 1.92 ppm, 3.84 ppm, and 7.68 ppm, following a doubling dose escalation method.

Observations:

1. Within the first week, 4–5 mice exposed to doses of 3.84 ppm or higher died.
2. Mice exposed to 1.92 ppm survived for two weeks before showing signs of severe toxicity and death.
3. Mice treated with lower doses (up to 0.96 ppm) survived for one month without severe adverse effects.

Based on these observations, 0.96 ppm was established as the maximum tolerated dose for Endosulfan and selected for further experimentation as a sub-lethal dose to assess its nephrotoxic effects.

Phase 2: Determination of Maximum Tolerated Dose (MTD) of Amla

To establish the MTD for Amla, aqueous extracts at concentrations of 2 mg/cc, 5 mg/cc, and 10 mg/cc were orally administered to separate groups of mice for one, two, and four weeks.

All mice treated with 10 mg/cc of Amla for one month survived without observable toxicity, establishing this as the MTD.

***Experimental Design*:** A total of 30 mice were randomly divided into three groups, with 10 mice per group:

***1. Control Group*:** Received normal feed and water.

***2. Endosulfan-Treated Group*:** Administered 0.96 ppm Endosulfan daily for 7, 14 or 28 days.

***3. Amla-Treated Groups*:** Following Endosulfan administration, the treated mice received 10 mg/cc of Amla daily for 7, 14 or 28 days respectively to assess its ameliorative effects.

Histopathological Examination:

Mice were anesthetized with chloroform to minimize stress during sacrifice at intervals of one, two, and four weeks post-treatment. Kidney tissues were excised, rinsed twice with normal saline, and cut into small pieces using a sharp blade on a clean glass surface.

The tissues were fixed in 10% neutral buffered formalin for at least 24 hours to preserve cellular integrity. After fixation, samples were washed under running water for 24 hours, dehydrated with graded alcohol, cleared in xylene, and embedded in paraffin to prepare tissue blocks. Thin sections of 3–4 µm were cut using a microtome and stained with Hematoxylin and Eosin for histopathological evaluation.

Histological Assessment

The stained kidney sections were examined under a light microscope to assess structural changes in renal corpuscles and tubules. Parameters such as necrosis, vacuolation, and clumping of glomeruli were documented. Changes in the dimensions of Bowman's capsule, glomeruli, proximal convoluted tubules (PCT), and distal convoluted tubules (DCT) were measured using a calibrated ocular micrometer. Additional quantitative assessments included:

1. Comparison of renal structural dimensions across groups.
2. Documentation of ameliorative effects, such as reductions in necrotic lesions and improvements in brush border integrity.

Ethical Statement: All experimental procedures were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by the Institutional Animal Ethics Committee of Patliputra University.

RESULTS

Histological Examination of Renal Corpuscles and Tubules

Histological analysis of the renal cortex and associated tubules was conducted using light microscopy, as these regions exhibited pronounced histopathological changes in both Endosulfan-treated and Amla-treated groups. Measurements of Bowman's capsule, glomeruli, proximal convoluted tubules (PCT), and distal convoluted tubules (DCT) dimensions were recorded to quantify structural alterations.

In the **control group** (Figure 1), kidney tissue displayed normal histological features, with no significant pathological lesions. The Bowman's capsule, glomeruli, PCT, and DCT retained their typical structure and function, with no observable signs of necrosis, vacuolation, or inflammation.

In the **Endosulfan-treated group** (Figure 2), histopathological changes were apparent, increasing with the duration of exposure:

- After **7 days** of Endosulfan administration at 0.96 ppm, minor histological changes were observed, with early signs of necrosis and mild vacuolation in the renal corpuscles.
- After **14 days**, the lesions became more pronounced. There was clumping of the glomeruli, increased necrosis, and thickening of the Bowman's capsule.
- After **28 days**, the damage was extensive. The glomeruli appeared wire-like due to acellularity, and the Bowman's capsule showed significant necrosis. Vacuolation and eosinophilic cellular infiltrates were prominent in both the PCT and DCT.

Quantitative analysis revealed significant reductions in the dimensions of key renal structures (**Table 1 and Figure 4**):

- After 7 days of Endosulfan administration at 0.96 ppm, Bowman's capsule dimensions decreased by -13.40%, which was highly significant ($P \leq 0.001$). Glomerular dimensions decreased by -21.23%, also highly significant ($P \leq 0.0001$). PCT dimensions decreased by -18.73% ($P \leq 0.001$), and DCT dimensions decreased by -15.65% ($P \leq 0.001$).
- After 14 days of Endosulfan administration at 0.96 ppm, Bowman's capsule dimension decreased by -17.77%, which was highly significant ($P< 0.0001$). Glomerular dimension decreased by- 34.20%, also highly significant ($P<0.00001$). PCT dimension decreased by-24.73%, also highly significant($P<0.00001$). DCT dimension decreased by-18.70%, also highly significant ($P<0.0001$).
- After 28 days of Endosulfan administration at 0.96 ppm, Bowman's dimension decreased by 23.64%, ($P<0.00001$), Glomerular dimension decreased by-39.62%, ($P<0.00001$), PCT dimension decreased by -32.86% ($P<0.00001$), DCT dimension decreased -25.22%, ($P<0.0001$).

These findings confirm that prolonged Endosulfan exposure leads to severe histological abnormalities in the renal cortex, reflecting impaired kidney function.

Ameliorative Effects of Amla Treatment

In the **Amla-treated group** (Figure 3 and Figure 4), the administration of 10 mg/cc Amla daily for 7, 14, and 28 days demonstrated noticeable ameliorative effects on Endosulfan-induced nephrotoxicity:

After **7 days**, minor improvements were noted. While necrosis in the Bowman's capsule was reduced, the changes in dimensions of renal structures were not statistically significant: Bowman's capsule (+4.36%, **$P > 0.05$**), glomeruli (+6.59%, **$P > 0.05$**), PCT (+6.52%, **$P < 0.05$**), and DCT (+5.15%, **$P > 0.05$**) (**Table 1 and Figure 4)**.

After **14 days**, the renal tissue showed further recovery. The necrotic lesions were reduced, vacuolation was diminished, and the brush border of the PCT lumen was restored. The changes in dimensions were more significant: Bowman's capsule (+7.82%, **$P < 0.05$**), glomeruli (+11.98%, **$P < 0.01$**), PCT (+12.17%, **$P < 0.001$**), and DCT (+9.79%, **$P < 0.05$**) (**Table 1 and Figure 4)**.

After **28 days**, the renal tissue exhibited near-normal histology, with minimal signs of necrosis and improved cellular integrity. The lumen of the PCT appeared normal with a well-defined brush border, and the DCT lumen appeared sharp. Significant dimensional increases were observed (**Table 1 and Figure 4): Bowman's capsule:** +22.29% (**$P \leq 0.00001$**), **Glomeruli:** +46.48% (**$P \leq 0.00001$**), **PCT:** +38.42% (**$P \leq 0.0001$**), **DCT:** +22.67% (**$P \leq 0.001$**)

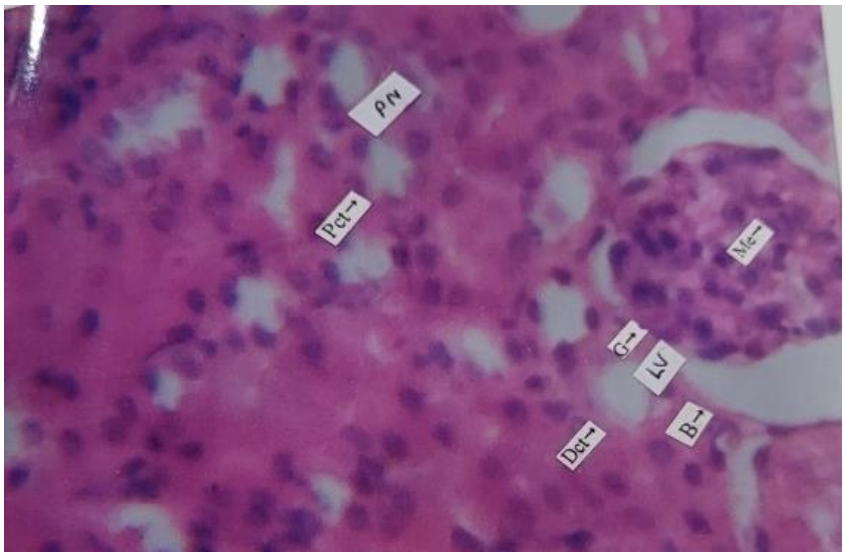

Figure 1: Normal Kidney tissue showing normal G (G), Bowmans capsule(B), Proximal convoluted tubule(Pct), Distal convoluted tubule (Dct), Mesangial cells (Me), Podocyte Nuclei (PN). (200X)

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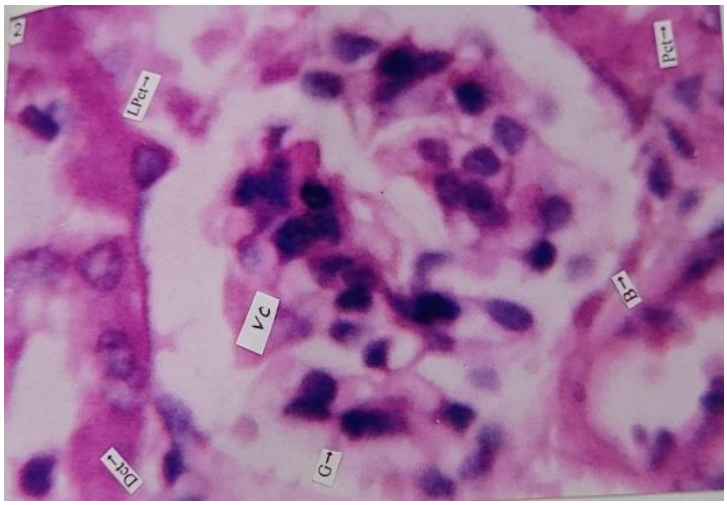

Figure 2: Kidney tissue from Endosulfan-treated mice showing pycnotic nuclei, vacuolation (Vc), and necrosis, abnormalities in Glomerulus (G) due to acellularity, damaged leaky Pct (LPct), Dct. Bowmans capsule(B), Proximal convoluted tubule(Pct), Distal convoluted tubule (Dct) (400X)

Table 1: Amla (10 mg) Induced Changes in Dimensions of Bowman's Capsule, Glomerulus, Proximal Convoluted Tubules (PCT), and Distal Convoluted Tubules (DCT) in Endosulfan-Treated Mice (Values Expressed in µm). Mice were treated with 0.96PPM Endosulfan for 7 days (EN7), 14 days (EN14) or 28 days (EN28).

	BOWMANS CAPSULE			GLOMERULUS			PCT			DCT		
	% CHANGE	MEAN	P	% CHANGE	MEAN	P	% CHANGE	MEAN	P	% CHANGE	MEAN	P
Control		66.6			42.4			28.3			23	
EN7	-13.4	57.5	***	-21.23	33.4	****	-18.73	23	****	-15.65	19.4	***
EN7 +Amla	5.22	60.5	*	2.99	34.4	NS	10.43	25.4	***	13.4	22	**
EN14	-17.77	54.6	****	-34.2	27.9	*****	-24.73	21.3	*****	-18.7	18.7	****
EN14 +Amla	10.81	60.5	***	19.35	33.3	**	22.54	26.1	****	16.58	21.8	***
EN28	-23.64	50.7	*****	-39.62	25.6	******	-32.86	19	*****	-25.22	17.2	****
EN28 +Amla	22.29	62	*****	46.48	37.5	*****	38.42	26.3	****	22.67	21.1	***

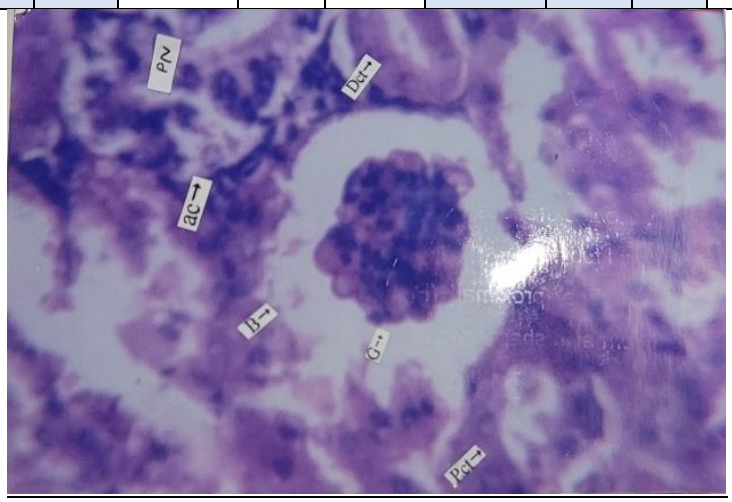

Figure 3: Structure, reduced necrosis, and improved cellular integrity. Glomerulus(G), Bowmans capsule(B), Proximal convoluted tubule(Pct),Distal convoluted tubule (Dct), Podocyte Nuclei (PN). (200X)

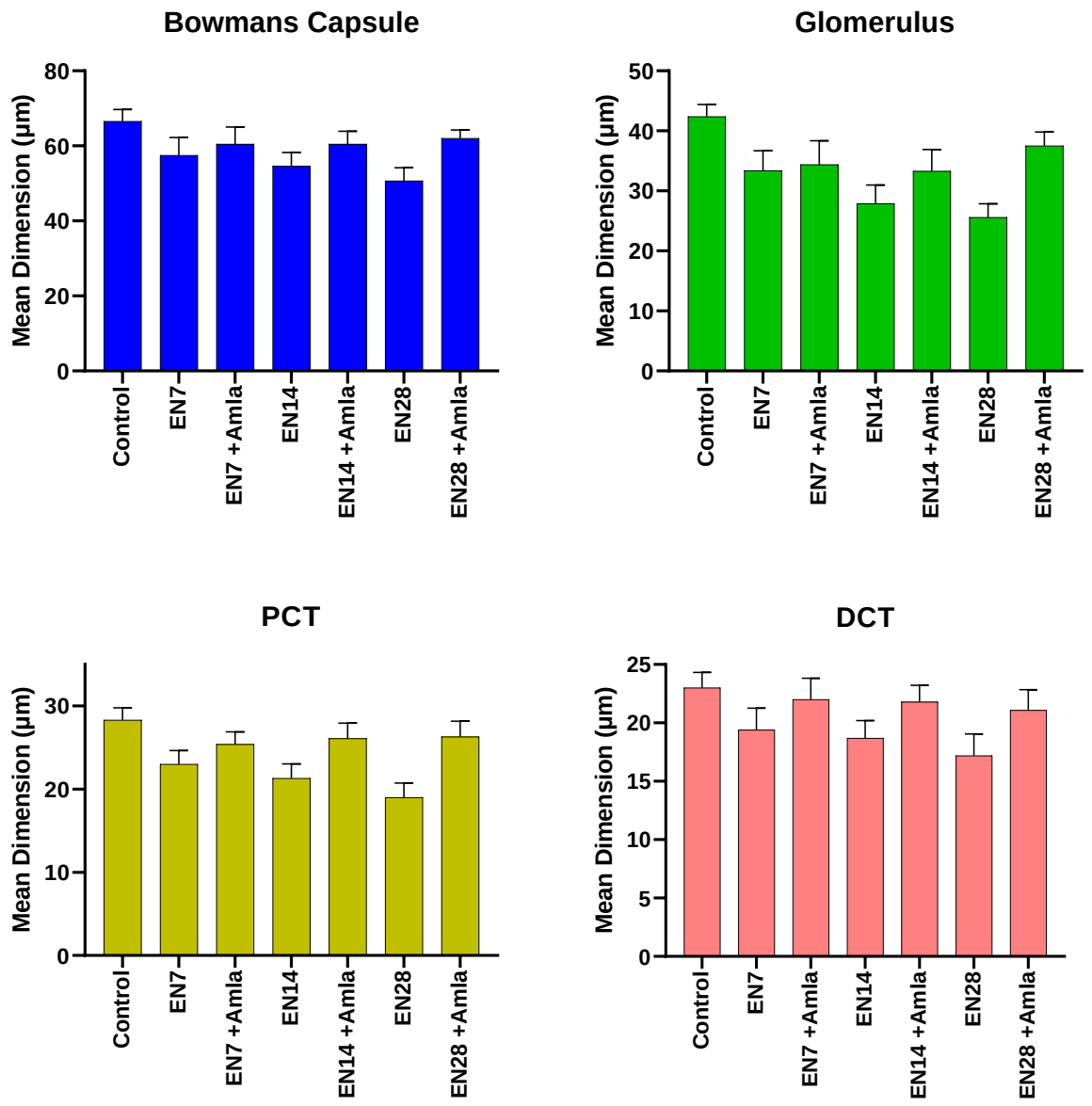

Figure 4: Amla (10 mg) Induced Changes in Dimensions of Bowman's Capsule, Glomerulus, Proximal Convoluted Tubules (PCT), and Distal Convoluted Tubules (DCT) in Endosulfan-Treated. Mice were treated with 0.96PPM Endosulfan for 7 days (EN7), 14 days (EN14) or 28 days (EN28). Error bars represent standard deviation of the mean values.

The results confirm that Endosulfan administration induces significant nephrotoxic effects, with pronounced histological changes in the renal corpuscles and tubules. Treatment with Amla effectively mitigated these effects, reducing necrotic lesions, restoring cellular structure, and improving the dimensions of renal components. These findings highlight the potential of Amla as a natural nephroprotective agent against pesticide-induced renal damage.

DISCUSSION

The findings of this study demonstrate that sublethal exposure to Endosulfan induces significant histological changes in the renal cortex and tubules of Swiss albino mice. These results align with earlier research highlighting the nephrotoxic effects of Endosulfan, including cellular necrosis, glomerular clumping, and vacuolation (Khan, 2014). Prolonged exposure, as observed in this study, exacerbates these effects, leading to structural damage such as wire-like glomeruli and complete acellularity in the visceral and parietal layers (Figure 2). The severity of these changes underscores the impact of Endosulfan on renal function, likely driven by oxidative stress and inflammation.

Mechanisms of Endosulfan-Induced Nephrotoxicity: Endosulfan's toxic effects on the kidney are thought to result from its ability to generate reactive oxygen species (ROS), causing oxidative stress. ROS destabilize cellular membranes, damage DNA, and impair enzymatic functions, leading to necrosis and apoptosis. Similar findings have been reported in studies demonstrating ultrastructural damage in mice kidneys due to Endosulfan exposure (Caglar *et al.,* 2003). Furthermore, the pesticide's disruption of drug-metabolizing enzymes in renal tissue has been documented (Singh and Pandey, 1980), which likely compounds its nephrotoxic potential.

Protective Effects of Amla: The administration of *Emblica officinalis* (Amla) following Endosulfan exposure significantly alleviated nephrotoxicity, as evidenced by improved histological integrity of renal tissues (Figure 3). The restoration of glomerular structure, reduction in necrosis, and normalization of tubular dimensions indicate the efficacy of Amla in mitigating pesticide-induced damage. The nephroprotective effects of Amla may be attributed to its high antioxidant content, particularly vitamin C, polyphenols, and flavonoids, which help counteract oxidative stress and inflammatory responses induced by endosulfan. The histopathological improvements observed in Amla-treated mice further substantiate its protective role in maintaining renal structure and function.

Naik *et al.,* (2005) have reported Triphala's ability to donate electrons to stabilize ROS without becoming free radicals themselves, thereby protecting cellular structures. This mechanism aligns with the observed reduction in histopathological lesions, including vacuolation and polymorphic nuclei, in Amla-treated mice. The findings of this study corroborate earlier research by Kavutcu *et al.,* (1996), which demonstrated Amla's capacity to enhance enzymatic defense mechanisms and restore renal function in gentamicin-induced nephrotoxicity.

Comparative Studies: Various plant-derived compounds have been shown to exert nephroprotective effects against different nephrotoxic agents. For instance, gum Arabic extract has been reported to mitigate carbon tetrachloride-induced nephrotoxicity (Habashy, N. H., 2024), while chicory and artichoke leaf extracts have demonstrated renoprotective effects in similar toxicant models (Eassawy, M. M., 2024). Additionally, onion and purple cabbage extracts have been found to reduce renal damage associated with carbon tetrachloride exposure (Arooj Jamshaid, 2023). Although carbon tetrachloride is an organochlorine compound, endosulfan is more commonly used as an agricultural pesticide, necessitating investigations into its specific toxic effects and possible mitigation strategies.

Several studies have highlighted the therapeutic potential of Amla in counteracting oxidative damage induced by environmental toxicants. Manish *et al.,* (2014) documented the efficacy of crude Amla extract in mitigating arsenic-induced oxidative stress, a condition similar to the oxidative damage caused by Endosulfan. Similarly, (Bhattacharya *et al.,* 1999) reported the protective effects of Amla's tannoid principles, which function as free radical scavengers, in various toxicological models. Additionally, (Tamadon *et al.,* 2015) emphasized the role of antioxidants in managing chronic kidney disease, further validating the application of Amla in reducing oxidative stress and histopathological damage. The findings of this study are consistent with these observations, demonstrating that Amla not only mitigates cellular damage but also restores structural and functional integrity in the kidney.

Unlike previous studies that primarily focused on nephrotoxicity induced by chemotherapeutic agents such as cisplatin (Purena et al, 2018) or other toxicants like malachite green (Sinha *et al.,* 2021), this study is the first to establish Amla's nephroprotective potential against endosulfan toxicity in a mammalian model. This

distinction is crucial, as endosulfan remains one of the most extensively used pesticides in agricultural settings, posing a significant risk to human health through environmental and occupational exposure.
Implications and Recommendations: The protective role of Amla observed in this study underscores its potential as a natural antioxidant for managing pesticide-induced nephrotoxicity. Given its availability and safety, Amla could be a viable therapeutic agent for populations exposed to organochlorine pesticides such as Endosulfan. The results also highlight the importance of incorporating antioxidants into dietary regimens to counteract environmental toxicants and reduce the risk of chronic kidney damage.

CONCLUSION

The study confirms that Endosulfan exposure provokes significant histological changes in renal tissue, leading to necrosis, glomerular clumping, and vacuolation. The administration of Amla effectively alleviates these effects, restoring normal histological structure and reducing oxidative stress. Moreover, these findings are particularly relevant to human health, considering the potential for pesticide exposure in agricultural communities and through contaminated food and water sources. The promising renoprotective properties of Amla suggest its potential as a natural dietary intervention to mitigate endosulfan-induced renal toxicity.
Further research is recommended to explore the molecular pathways involved in Amla's protective effects and to validate these findings in other models. Additionally, long-term studies could investigate the potential of Amla as a preventive dietary supplement for populations at risk of pesticide exposure.

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Ethical Statement: All experimental procedures were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by the Institutional Animal Ethics Committee of Patliputra University.

CONFLICT OF INTEREST

The author declares no conflicts of interest concerning the research, authorship, or publication of this manuscript.

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