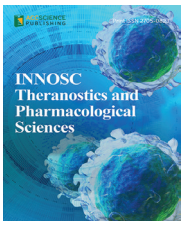
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RESEARCH ARTICLE

# Evaluation of Nephroprotective Effects of *Lawsonia inermis* Bark Extract: A Biochemical Approach

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## Abstract:

Gentamicin is atypical aminoglycoside medication used to treat bacterial infections. Gentamicin-induced nephrotoxicity is classified as a tubulopathy, in which tubular damage and destruction most frequently cause renal insufficiency. Our study aims to evaluate the nephroprotective effects of *Lawsonia inermis* bark extract against gentamicin-induced nephrotoxicity in rats. Animals were divided into four groups ($n$ = 6). Group I served as a normal control group, group II served as a gentamicin control group, and in Groups III and IV, gentamicin-challenged animals were treated with *L. inermis* bark extract at doses of 100 mg/kg and 200 mg/kg, respectively. Based on the results, we found that treatment with *L. inermis* bark extract at doses of 100 mg/kg and 200 mg/kg significantly reduced the elevated creatinine and urea in the blood. Besides, the extract also improved the total protein level in the blood. It is well known that gentamicin intoxication reduces antioxidant defense, so we examined the levels of endogenous antioxidants (superoxide dismutase, catalase, and reduced glutathione) and discovered that the extract at doses of 100 mg/kg and 200 mg/kg significantly improved the activity of these antioxidants. Hence, we can conclude that the nephroprotective potential of *L. inermis* bark extract may be attributed to its antioxidant activity.

**Keywords:** Nephroprotective, *Lawsonia inermis,* Gentamicin, Creatinine, Urea, Total protein, Antioxidants, Superoxide dismutase, Catalase, Reduced glutathione

## 1. Introduction

Acute renal damage refers to a sudden decline in renal excretion capacity. The abrupt impairment of kidney ability that typically returns after few weeks or months is referred to as acute nephro-insufficiency [1]. Structural and functional operations of several systems, including those of the human kidneys, cardiac, and hepatic systems, can be significantly altered by external toxins, pharmaceuticals, and medications like antibiotics. Nonetheless, due to the kidneys' innate abilities to eliminate medications like antibiotics, they have become the main component implicated in acute renal disease [2]. Gentamicin is a typical aminoglycoside drug used to treat bacterial infections. Gentamicin-induced kidney toxicity is categorized as a tubulopathy, where renal insufficiency is most usually brought on by tubular damage and destruction, which may lead to the clinical findings of proteinuria, electrolytic changes, and enzymuria. It has been proposed by a number of experimental findings that the development of renal injury may be accelerated by

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oxidative radical-induced damage. Another factor is that pro-inflammatory cytokines have been found to significantly impact the development of renal damage brought on by gentamicin [3].

*Lawsonia inermis* is a therapeutic herb that is still used today. It has been used for many years as both a cosmetic and a medical source. Besides, it is frequently prescribed as a mild laxative and emergency contraceptive, as well as an astringent in traditional African holistic therapies. *L. inermis* also shows several therapeutic benefits, including analgesic properties, reducing blood sugar, keeping the liver healthy, boosting the immune system, preventing cancer, reducing inflammation, and inhibiting the growth of bacteria, and viruses, fungi, trypanosomes, and plasmodium [4]. Phytochemical screening of both ethanol and aqueous extracts of the leaves of *L. inermis* showed various constituents such as alkaloids, carbohydrates, resins, saponins, flavonoids, coumarins, and steroids [4], which suggests that it may possess nephroprotective activity. However, there are no reports on its neha protective activity. Hence, in this study, an attempt was made to access the potentiality of *L. inermis* bark extract against gentamicin-induced renal damage in laboratory animals.

## 2. Methodology

### 2.1. Materials

Gentamicin ampoules were acquired from Jawa Pharmaceuticals, India. Biochemical kits such as serum creatinine and urea, and total protein test kits were purchased from Accurex Biomedical, India. All the other chemicals utilized during the experiment were analytical grade. The reagents required in this study were purchased from SRL Chemicals, India.

(i). Gentamicin
Brand Name: Avrocin (80 mg)
Manufacturers: Shinto Organics (P) Ltd.
Type: Injection

(ii). All kits
Creatinine and blood urea kits are obtained from Span Diagnostic, India.

### 2.2. Plant collection and extraction

Stems of the plants were gathered from rural areas, and then they were identified and kept in the herbarium on a herbarium sheet (which is usually 100% acid-free, archival, and is used for mounting herbarium specimens) with a specimen identification number.

Extraction was done in the following manner [5-7]:

- The stems were collected, and the bark was separated.
- The bark was then sliced into small pieces, and the pieces were dried in a shaded area.
- Afterward, the dried bark pieces were pulverized and the powder (100 g) was kept in petroleum ether (60 – 80°C, laboratory grade) for 72 h with intermittent shaking to remove the plant lipids.
- The solution was then filtered and the powder was subjected to ethanol (99%) for a week with intermittent shaking.
- Filtration was done, and the filtrate was collected in a beaker and concentrated in a rotary evaporator to get a brown-colored, viscous mass of extract (5.60% w/w), which was then refrigerated at 4°C for further use.

### 2.3. Experimental animals

Wistar rats (male, 160 – 180 g) were used in this study. The animals were kept in polypropylene cages with an optimum supply of food (pellets) and drinking water. All the experimental work on animals was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and as per the animal ethics guidelines of the institution (Ref. no. F4/CIPT/ADMN/2020-21/008). All the animals were acclimatized for a week before the commencement of the experiment.

### 2.4. Acute oral toxicity

Acute oral toxicity was performed following Organization for Economic Co-operation and Development(OECD) 423 recommendations. Animals were administered 2000 mg/kg of the extract, and for the first 30 min and then for 4 h, they were carefully observed to see if there was any toxicity in terms of changes in cardiac physiology, neurobehavioral physiology, or respiration pattern. Afterward, animals were kept in observation for 24 h and then for 2 weeks to assess if there was any toxicity [8].

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### 2.5. Treatment protocol

Animals were grouped into four groups ($n = 6$):

(i). Group I: Normal control (received 0.30% carboxymethyl cellulose).

(ii). Group II: Nephrotoxic animals (received only gentamicin at the dose of 100 mg/kg, intraperitoneal [i.p.] for 8 days).

(iii). Group III: Received *L. inermis* (100 mg/kg; p.o.) after 1 h of gentamicin (100 mg/kg; i.p.) administration, for 8 days.

(iv). Group IV: Received *L. inermis* (200 mg/kg; per os [p.o.]) after 1 h of gentamicin (100 mg/kg; IP) administration for 8 days.

After the last dose of *L. inermis* or gentamicin, animals were kept for the night fasted, and on the 9$^{th}$ day, blood was collected from anesthetized animals (animals were anesthetized using thiopental sodium at a dose of 40 mg/kg; i.p.) for biochemical estimation of serum creatinine and urea levels. On the other hand, the concentration of endogenous antioxidant enzymes (superoxide dismutase, catalase, and reduced glutathione) in the blood was also estimated [9].

### 2.6. Data analysis

Data analysis was performed using GraphPad Prism software. The mean standard error was used to represent the data. The method used was one-way analysis of variance (ANOVA) with Tukey's multiple comparisons. $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Acute oral toxicity

No sign of oral toxicity or mortality was found at the dose of 2000 mg/kg of *L. inermis* bark extract. Hence, 100 mg/kg (1/20$^{th}$) and 200 mg/kg (1/10$^{th}$) doses were used in this study.

### 3.2. Effects of *L. inermis* bark extract on biochemical parameters

**Table 1** represents the biochemical data such as levels of serum creatinine and urea acquired from the different groups. We observed that the serum creatinine level in Group II (only gentamicin-treated animals) was significantly higher when compared to normal and other groups. Animals in Groups III and IV treated with the *L. inermis* bark extract (100 and 200 mg/kg) demonstrated a noteworthy decrease in the serum creatinine concentration when contrasted with only gentamicin-intoxicated animals in Group II. Similarly, we found that the serum urea level was higher in the animals in Group II compared to other groups. Animals treated with the *L. inermis* bark extract (100 mg/kg and 200 mg/kg) illustrated a noteworthy decrease in the serum urea level in Groups III and IV, respectively, compared to only gentamicin-treated animals (Group II). Treatment with the extract at a dose of 100 mg/kg and 200 mg/kg enhanced the serum total protein concentration, respectively, in Groups III and IV.

**Figure 1** represents the results of *L. inermis* bark extract (100 and 200 mg/kg) on the serum concentration of endogenous antioxidants (superoxide dismutase, catalase, and reduced glutathione). From **Figure 1**, we found that the animals treated with gentamicin showed a marked fall in the serum concentration of endogenous antioxidants. Especially the Group II rats only treated with gentamicin showed a substantial decrease in superoxide dismutase, catalase, and reduced glutathione levels when compared with the normal control group (Group I). Nonetheless, animals in Groups III and IV treated with *L. inermis* bark extract at doses of 100 mg/kg and 200 mg/kg,

**Table 1.** Effects of *L. inermis* extract on serum creatinine and urea in gentamicin-challenged animals

| Groups | Treatment | Creatinine (mg/dL) | Urea (mg/dL) |
|---|---|---|---|
| I | Normal control | 0.36±0.05 | 27.96±1.66 |
| II | Gentamicin (100 mg/kg) | 2.04±0.38$^{###}$ | 108.28±3.28$^{###}$ |
| III | Gentamicin (100 mg/kg) + *L. inermis* (100 mg/kg) | 1.02±0.13*** | 95.37±4.60*** |
| IV | Gentamicin (100 mg/kg) + *L. inermis* (200 mg/kg) | 0.68 ± 0.11*** | 63.18 ± 1.82*** |

Data were represented as mean±standard error. $^{###}P<0.001$ when group II versus group I and $^{***}P<0.001$ when Groups III and IV versus Group II.

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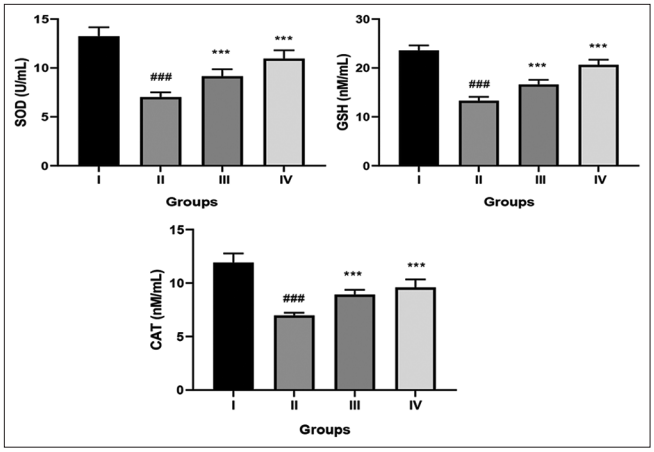



**Figure 1.** Data were represented as mean ± standard error. $^{###}P < 0.001$ when Group II versus Group I and $***P < 0.001$ when Groups III and IV versus Group II.
SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione.

respectively, demonstrated a notable improvement in the levels of superoxide dismutase, catalase, and reduced glutathione in the serum.

## 4. Discussion

Nephrotoxicity due to gentamicin administration in animals is very well documented. Several studies have shown that gentamicin's nephrotoxic effects are caused by an increase in oxidative assaults or increased production of reactive radicals such as superoxide anions and hydroxyl radicals, which cause renal tubular cell necrosis and acute kidney failure [10]. High oxidative stress or increased production of reactive oxygen species causes DNA breakdown, membrane lipid peroxidation, and a decrease in cellular respiration activity, as well as inhibition of the electron transport chain, resulting in cell death (through apoptosis or necrosis) [11]. For such reasons, in the present experiment, a gentamicin model was selected to study the effect of *L. inermis* bark extract on gentamicin-intoxicated animals.

Based on the results shown in **Table 1**, we found that there was an obvious increase in the serum creatinine and urea levels and a decrease in the total protein in gentamicin-intoxicated animals. Treatment with *L. inermis* bark extract at the doses of 100 and 200 mg/kg illustrated a notable improvement in the concentration of the aforementioned biochemical parameters, which indicates that the bark extract has enough renoprotective potential against gentamicin-induced nephrotoxicity.

Overproduction of reactive oxygen radicals decreases the activity of superoxide dismutase, catalase, and reduced glutathione. Reduced glutathione is considered a first-line defender against oxidative stress because it reduces the production of hydroxyl radicals from hydrogen peroxide. Besides, the catalase enzyme scavenges free radicals and detoxifies hydrogen peroxide. Furthermore, superoxide dismutase is an enzyme that reduces the fabrication of oxygen-free radicals and protects our vital organs from oxidative stress [12,13].

In our present study, we found that when compared to the normal control group (Group I), Group II in particular demonstrated a noteworthy drop in the activities of superoxide dismutase, catalase, and reduced glutathione. But when Groups III and IV animals were given *L. inermis* bark extract at doses of 100 and 200 mg/kg, correspondingly, the levels of catalase, superoxide dismutase, and reduced glutathione in the blood increased significantly. In the current study, ethanol extract of the leaves of *L. inermis* (100 mg/kg and 200 mg/kg body weight [b.w.]) has shown dose-dependent nephroprotective effect which may be due to the presence of phytochemicals present in the extract which protected the kidneys from the damage induced by gentamicin. As reported it is rich in flavonoids and plyphenolics which are great antioxidants and may contribute to a great extent to the nephroprotective effects of this plant.

A previous study of *L. inermis* against carbon tetrachloride-induced hepatotoxicity in animals showed that this plant considerably increased reduced glutathione levels. This incident illustrates the antioxidant protection of *L. inermis* against toxicants that induce organ damage [14]. Besides, *L. inermis* showed antioxidant effects against 2-acetylamino-fluorene-induced hepatotoxicity in vivo [15]. In our study, we found that *L. inermis* bark extract showed a notable antioxidant effect which may be responsible for nephroprotection against gentamicin-induced nephrotoxicity in rats.

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## 5. Conclusion

The results of our experiment demonstrated that *L. inermis* bark extract has a protective role against gentamicin-induced nephrotoxicity in rats. The antioxidant defense of the extract may be the valuable outcome of this experiment. However, further studies are needed to evaluate the possible molecular protective mechanisms of *L. inermis* bark extract involved in gentamicin-induced nephrotoxicity.

The toxicity of *L. inermis* extract was reported in several *in vivo* studies which all reported its safety on ingestion. In a study, it was discovered that the tissue samples from control animals' liver, kidney, heart, and spleen were virtually normal after the ingestion of *L. inermis* extract. In addition, the tissues of the mice that received extract doses of 200 g/kg and 500 g/kg b.w. did not exhibit any lesions (pathological alterations). The conclusion of this study is also consistent with other studies in terms the biochemical and hematological response in albino Wister rats treated with *L. inermis* aqueous extract. Hence, consuming a 500 mg/kg b.w. dose of an 80% ethanol extract of *L. inermis* has no negative effects on the tissues of the organs tested in rats. However, more investigation is recommended to study its effects on a subcellular level [16].

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## Conflict of interest

No conflict of interest was declared by the authors.

## Author contributions

*Conceptualization:* Sayeed Mohammad Firdous, Marwa A.A.Fayed
*Data curation:* Marwa A.A.Fayed
*Formal analysis:* Marwa A.A.Fayed
*Methodology:* Sayeed Mohammad Firdous
*Supervision:* Sayeed Mohammad Firdous
*Writing – original draft:* Marwa A.A.Fayed
*Writing – review & editing:* Sayeed Mohammad Firdous, Marwa A.A.Fayed

## Ethics approval and consent to participate

All the experimental work on animals was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and as per the animal ethics guidelines of the institution (Ref. no. F4/CIPT/ADMN/2020-21/008).

## Consent for publication

Not applicable.

## Availability of data

Not applicable.

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