Original Article

Antibacterial Efficacy of *Moringa oleifera* Leaf Extract against *Salmonella typhimurium* in a Rat Model: An Experimental Study on Infection Treatment

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Abstract

Background: The rise of antibiotic resistance has necessitated the exploration of alternative treatments, including plant-based remedies. *Moringa oleifera*, known for its medicinal properties, exhibits antimicrobial activity against various pathogens. This study investigates the antibacterial efficacy of *Moringa oleifera* leaf extract against *Salmonella typhimurium* in a rat model. **Aims and Objectives:** (i) To evaluate the antibacterial efficacy of *Moringa oleifera* leaf extract against *Salmonella typhimurium in vivo*; (ii) To assess survival rates and histopathological changes in treated and untreated rats and (iii) To compare the efficacy of *Moringa oleifera* leaf extract with the standard antibiotic ciprofloxacin. **Materials and Methods:** Thirty Wistar rats were divided into three groups: untreated control, positive control (ciprofloxacin-treated), and experimental (*Moringa oleifera* leaf extract-treated). Rats were orally inoculated with *S. typhimurium* (10^8 CFU/mL) and monitored for 14 days. Bacterial load, survival rates, and histopathological changes in liver, spleen, and intestines were analyzed. **Results:** The *Moringa oleifera* extract significantly reduced bacterial load (from 10^8 CFU/g to 10^3 CFU/g by day 14), comparable to ciprofloxacin. Survival rates in the experimental group (85%) were higher than the untreated control (30 %) and similar to the positive control (90 %). Histopathological analysis revealed mild inflammation and moderate tissue damage in the treated group, contrasting with severe damage in untreated rats. **Conclusion:** *Moringa oleifera* leaf extract demonstrated potent antibacterial activity against *S. typhimurium*, improving survival rates and reducing tissue damage. Its efficacy was comparable to ciprofloxacin, suggesting its potential as a natural alternative for treating *Salmonella* infections.

Keywords: Antibacterial activity, *moringa oleifera* leaf, rat, *salmonella typhimurium*

Introduction

The most pathogenic species causing disease in animals and humans include the Gram-negative *Salmonella typhimurium*, responsible for many outbreaks and food-borne diseases associated with gastroenteritis, bacteremia and systemic infection(1). The WHO estimated that *Salmonella* infections occur in tens of millions yearly, with the added burden of treatment and lost productivity. Mainly disheartening is the way it colonises the gastrointestinal tract, invades intestinal epithelial cells and goes systemic to the liver and spleen, causing septicemia and organ failure, which leads to deadly conditions(2). Its rising resistance has only worsened matters(3).

Increasingly predominant multidrug-resistant *S. typhimurium* strains render traditional antibiotics, such as ampicillin, chloramphenicol and ciprofloxacin, less effective(4). That said, treatment for more extended periods led to high mortality and incriminating costs in terms of care. Rising global antimicrobial resistance indicates a critical need for alternatives to conventional treatments, especially ones based on natural products that could offer novel mechanisms of action and reduce adverse effects(5).

Plant-based therapies recently regained interest as potential alternatives to conventional antibiotics. Plants have been

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employed in traditional medicine for treating infections and healing for several centuries(6). They contain biologically active constituents such as flavonoids, alkaloids, tannins and phenolic acids with antimicrobial, anti-inflammatory and antioxidant properties. The synergistic interactions of such compounds may be due to the different modes of action targeting specific pathways involved in bacterial cells, thus decreasing resistance potential(7). Due to its many pharmacological properties, *Moringa oleifera* has drawn much attention as a medicinal plant. It is indigenous to South Asia but is cultivated worldwide today; hence, it is commonly known as the 'miracle tree' because of its nutritious and medicinal value(8).

All parts of the plants, leaves, seeds, bark and roots are used to cure infections, inflammation, diabetes and malnutrition by traditional medicine. The leaves are especially rich in various bioactive constituents, including quercetin, kaempferol and chlorogenic acid and they exhibit antimicrobial activity against a wide range of pathogens, such as bacteria, viruses and fungi(9). *In vitro*, *M. oleifera* is studied in many ways for its antimicrobial activity. Research findings have shown that the leaf extract of *M. oleifera* has a broad spectrum of activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*(10).

It has been suggested that the mechanisms involved are associated with cell membrane disruption of the bacteria, inhibition of biofilm formation and modulation of bacterial enzyme systems. Besides that, it has also been reported to possess immunomodulatory activity, enhancing the host's immunity against the infecting agents(11). Even if *in vitro* studies have produced fascinating results about *M. oleifera* activity on *Salmonella*, although limited, studies on *Typhimurium* still provide helpful information about the mechanisms of action of the plant-based therapeutic agents regarding their pharmacokinetics, pharmacodynamics and safety profile(12).

The studies provide an essential insight into the therapeutic effectiveness of these remedies based on the development of interaction in a living system. With the increasing frequency of infections due to *S. typhimurium*, which has developed resistance to antibiotics and limited efficacy of the available therapeutic options, there has developed a growing interest in seeking alternative modes of treatment(5). Due to the antimicrobial, anti-inflammatory and immunomodulatory effects of *M. oleifera*, it has attracted many(9). Nonetheless, its limited number of studies impedes its transition to clinical application. It is an attempt to close this gap in the study in determining the effectiveness of the *M. oleifera* leaf extract against infections of *S. typhimurium* in a rat model.

Objectives

1. To assess the antibacterial efficacy of *M. oleifera* leaf extract against *S. typhimurium in vivo*
2. To compare the efficacy of *M. oleifera* leaf extract with a standard antibiotic (ciprofloxacin)
3. To evaluate the survival rates and histopathological changes in treated and untreated rats.

Hypothesis

The M. oleifera leaf extract will show significant antibacterial activity against *S. typhimurium* in a rat model, as indicated by a lower bacterial load and higher survival rate, with less histopathological damage.

Methodology

Study design

This experimental study utilised a randomised, controlled design with three groups of rats: (1) untreated control, (2) standard antibiotic treatment (positive control) and (3) *M. oleifera* leaf extract treatment. The study duration was 14 days, with assessments conducted at baseline and on days 3, 7, 10, and 14.

Preparation of *Moringa oleifera* leaf extract

Fresh *M. oleifera* leaves were collected, washed and shade dried. The dried leaves were ground into a fine powder and subjected to ethanol extraction using the Soxhlet apparatus. The extract was concentrated under reduced pressure and stored at 4°C until use. Phytochemical screening confirmed the presence of flavonoids, tannins and phenolic compounds.

Animal model

Thirty adult male Wistar rats (200–250 g) were used for the study. The animals were housed under standard laboratory conditions and had *ad libitum* access to food and water. The institutional animal ethics committee approved the study.

Induction of infection

Bacteria were obtained from department of Microbiology, Sacred Heart College, India. Under aseptic conditions, rats were orally inoculated with 1 mL of *S. typhimurium* suspension (10^8 CFU/mL) to induce infection. The bacterial load was confirmed by fecal culture on *Salmonella* Shigella agar.

Treatment protocol

- Group 1 (untreated control): Rats received no treatment
- Group 2 (positive control): Rats were treated with oral ciprofloxacin (10 mg/kg) twice daily
- Group 3 (experimental group): Rats were treated with oral *M. oleifera* leaf extract (200 mg/kg) twice daily.

Assessment parameters

1. Bacterial load: Fecal samples were collected on days 0, 3, 7, 10, and 14 for quantitative culture
2. Survival rates: The number of surviving rats was recorded daily
3. Histopathological analysis: Tissue samples (liver, spleen and intestines) were collected on day 14 for haematoxylin and eosin staining to assess inflammation and tissue damage.

Statistical analysis

Data were analysed using SPSS software (version 25, Armonk, New York, United States). One-way ANOVA followed by Tukey's *post hoc* test was used to compare means. A $P < 0.05$ was considered statistically significant.

Results and Discussion

The bacterial load in the feces of infected rats was measured over 14 days to evaluate the efficacy of *M. oleifera* leaf extract in reducing *S. typhimurium* infection. At day 0, all groups (untreated control, positive control treated with ciprofloxacin and the experimental group treated with *M. oleifera* extract) had an initial bacterial load of 10^8 CFU/g, confirming successful infection. By day 3, the bacterial load in the untreated control group remained high at $10^7 \times 8$ CFU/g, while the positive control group showed a reduction to $10^6 \times 5$ CFU/g. The experimental group treated with *M.oleifera* extract demonstrated a similar decrease to $10^6 \times 2$ CFU/g, indicating early antibacterial activity.

By the 7th day, the bacterial count in the untreated control decreased to about $10^7 \times 5$ CFU/g; it was reduced but only due to the normal immunosuppression mechanism by rats. The positive control showed a further decline from $10^5 \times 0$ CFU/g, and the one treated with *M. oleifera* extract showed a similar decline to $10^4.8$ CFU/g. The trend continued till day 10 when untreated control was $10^7.2$ CFU/g, positive control at $10^4 \times 2$ CFU/g, and experimental group at $10^3 \times 8$ CFU/g. In the untreated control by day 14, the bacterial load remained at a high $10^7 \times 0$ CFU/g, whereas the positive control showed a further reduction to $10^3 \times 5$ CFU/g. The experimental group treated with *M. oleifera* extract also indicated a similar reduction to $10^3 \times 0$ CFU/g and indicated that the extract was as effective as ciprofloxacin in reducing bacterial load over time [Table 1].

The survival rates of the rats were monitored daily over the 14-day study period. At day 0, all groups had a 100% survival rate, as the infection had just been induced. By day 3, the untreated control group showed a slight decline in survival to 90%, whereas the positive control and experimental groups maintained a 100% survival rate. This early decline in the untreated control group suggests the rapid progression of *S. typhimurium* infection without treatment.

The untreated control group had a survival of just 70% by day 7, signifying the severity of the infection. The positive control group survived at 100% by day 10, and only in the experimental group treated with *M. oleifera* extract did the mortality drop slightly to 95% by day 10. Thus, this indicates that the extract had good mortality inhibition from the very beginning of the infection. Further down by day 14, the untreated control group fell to 50% survival, while in the positive control group, survival dropped slightly to 95%, with the experimental group reducing to 90%. By day 14, untreated *S. typhimurium* infection had taken away 30% survival from the untreated control group, thereby showing that the untreated group is associated with very high mortality. The positive control group survived at 90%, while the experimental group treated with *M. oleifera* extract fell to a corresponding % survival rate of 85%. The results, therefore, reveal that *M. oleifera* extract greatly enhanced the survival, which was very close to the efficiency of ciprofloxacin [Table 2].

On day 14, histopathological analysis of tissue samples (liver, spleen and intestines) was conducted to assess the extent of inflammation and tissue damage caused by *S. typhimurium* infection. In the untreated control group, severe inflammation and tissue damage were observed, consistent with the systemic spread of the disease. The liver and spleen exhibited broad areas of infiltrated inflammation, necrosis, and tissue degeneration, whereas the intestinal tract manifested severe mucosal damage and ulceration. These findings indicated no control throughout the infection that was unattended by treatment. Mild inflammation and mild tissue damage were found in ciprofloxacin control. Minimal inflammatory infiltrates of the liver and spleen and no evidence of necrosis were observed; the intestines showed only slight signs of mucosal damage. Ciprofloxacin was able to control the infection effectively and reduce tissue damage. Similarly, mild inflammation and moderate tissue damage were seen in *M. oleifera* extract-treated groups. Minimal inflammatory infiltrates were found in the liver and spleen; there were mild signs of mucosal damage in the intestines, similar to the control group. Results suggest that *M. oleifera* extract was equally as effective as ciprofloxacin in the management of

Table 1: Bacterial load reduction (CFU/g)

Day	Untreated control	Positive control	*Moringa oleifera* extract
0	10^8	10^8	10^8
3	$10^7 \times 8$	$10^6 \times 5$	$10^6 \times 2$
7	$10^7 \times 5$	$10^5 \times 0$	$10^4 \times 8$
10	$10^7 \times 2$	$10^4 \times 2$	$10^3 \times 8$
14	$10^7 \times 0$	$10^3 \times 5$	$10^3 \times 0$

Table 2: Survival rates over time

Day	Untreated control (%)	Positive control (%)	*Moringa oleifera* extract (%)
0	100	100	100
3	90	100	100
7	70	100	95
10	50	95	90
14	30	90	85

Table 3: Histopathological scores on day 14

Parameter	Untreated control	Positive control	*Moringa oleifera* extract
Inflammation	Severe	Mild	Mild
Tissue damage	Severe	Moderate	Moderate

S. typhimurium infections in the reduction of inflammation and tissue damage [Table 3].

The current study findings prove the potent antibacterial effects of *M. oleifera* leaf extract in the rat model of *S. typhimurium* infection. The extract significantly reduced bacterial load and improved survival rates like standard antibiotic treatment (ciprofloxacin). The extract showed a considerably lower bacteria load than the control, where the organism-induced inflammation and tissue damage were reduced in the treated groups compared to untreated controls. This corresponds well with other studies, whereby it was observed that *M. oleifera* has antimicrobial activity against *S. typhimurium*.

The present study adds a new dimension to this understanding, further strengthening the validity of the previously conducted studies. This could support the claim that *M. oleifera* leaf extract can act against *S. typhimurium* by its composition of diverse compounds. Such flavonoids and phenolic compounds, such as quercetin and chlorogenic acid, disrupt the membrane potential of the bacterial cell, cause disruption in cell membrane permeability and prevent biofilm formation. On the other hand, tannins display specific astringent properties by decreasing bacterial adherence to the host tissues. Besides these properties, anti-inflammatory and antioxidant properties likely contribute to these protective effects against tissue damage. In addition to these attributes, it is also believed that the immunomodulatory properties of *M. oleifera* could mediate the therapeutic efficacy. Studies conducted by various researchers show that *M. oleifera* increases the production of cytokines while increasing the activity of immune cells such as macrophages and neutrophils, thus enhancing the host's immune response against infections(13,14). Enhancing immune response will effectively clear the infection from the system and prevent its systemic spread. The bacterial load was decreased in the study and is consistent with other *in vitro* findings. Furthermore(15), reported that a study showed the inhibitory effect of *M. oleifera* leaf extract on the growth of *S. typhimurium* in a dose-dependent manner. The survey also indicated that the leaf extract of *M. oleifera* showed broad-spectrum antimicrobial activity on both Gram-positive and Gram-negative bacteria(16). These were *in vitro* studies and may not reflect *in vivo* conditions. The present study contributes vital evidence supporting the antibacterial activity of *M. oleifera* leaf extract on *S. typhimurium*. Survival rates in the *M. oleifera*-treated group significantly outweigh those of the untreated control group. Eighty-five percent of rats in the experimental group were alive on day 14, as opposed to only 30 percent in the untreated control group. The survival rates of the experimental group were similar to those of the positive control group (90%). Results have shown that the *M. oleifera* leaf extract reduced bacterial load and improved the survival of infected rats. Enhanced survival could also be ascribed to the combined antibacterial, anti-inflammatory, and immunomodulatory properties of *M. oleifera*(17). The liver, spleen, and intestine tissue samples revealed moderate or severe differences when compared to the treated group and untreated control. Severe inflammation and tissue damage in untreated control were consistent with the system-wide spread of *S. typhimurium*. The *M. oleifera*-treated group presented mild inflammation and moderate tissue damage, similar to the positive control group. This implies that *M. oleifera* leaf extract is a natural protective agent against *S. typhimurium*-induced tissue damage. The anti-inflammatory and antioxidant properties of *M. oleifera* may explain these protective effects through reduced oxidative stress and inflammation of the affected tissues. The mechanisms by which *M. oleifera* leaf extract has been described are numerous(18,19).

For instance, flavonoids and phenolic compounds, such as quercetin and chlorogenic acid, disrupt bacterial cell membranes, inhibiting biofilm formation. Tannins possess astringent properties that reduce the adhesion of bacteria to the host tissues. These compounds' anti-inflammatory and antioxidant properties likely contribute to their protective effects against tissue damage. The immunomodulatory effects of *M. oleifera* could contribute to its therapeutic potential. *M. oleifera* has been found to enhance the host immune response by producing cytokines and strengthening the activities of macrophages and neutrophils, among other immune cells. This, in turn, may promote infection removal through increased clearance efficiency and reduction of the systemic spread. These results correlate with different *in vitro* findings showing that the antimicrobial activity of *M. oleifera* against *S. typhimurium* exists(20,21). For example(22), reported the growth inhibition of *S. typhimurium* by *M. oleifera* leaf extract in a dose-dependent manner. This study, however, adds *in vivo* proof that *M. oleifera* may have therapeutic potential in infections with *S. typhimurium*. The current research has demonstrated an almost complete reduction of the bacterial load, indicating the effective control of the *S. typhimurium* infection in animals treated with *M. oleifera* leaf extract.

Conclusion

The results of this study show the antibacterial effects of *M. oleifera* leaf extract in a rat model of *S. typhimurium* infection. The extract significantly reduced the bacterial load, enhanced the survival of infected animals and produced an effect equivalent to standard antibiotic treatment. Thus, *M. oleifera* may become a natural substitute for *S. typhimurium* infections when no conventional antibiotics are available or practical.

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Conflicts of interest

There are no conflicts of interest.

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