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Studies on Immunomodulatory Properties of *Loeseneriella Arnottiana* Wight Bark Extract

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ABSTRACT

Loeseneriella arnottiana, a liana (woody climber) belongs to Celastraceae family has been traditionally used for treating ailments such as diabetes. This study aimed to soxhlet extraction method to obtain an aqueous bark extract and evaluation of the immunomodulatory effects in *Rattus norvegicus* (Wistar albino strain). Rats were divided into five groups: a control group (water), a standard group (cyclophosphamide), and three treatment groups (T1, T2, T3) receiving 1, 2, and 3 mg/kg of the extract, respectively. All animals were immunized with sheep red blood cells (SRBCs) to elicit an immune response. Immunomodulatory activity was assessed through hemagglutination assays, histological analysis of immune organs and blood component evaluation. The hemagglutination assay revealed that the T2 group exhibited the highest antibody titer (1:2048), indicating a strong immune response. T1 showed a moderate response, while T3 had the weakest (1:1), suggesting a dose-dependent effect, with 2 mg/kg being the optimal dose. Histologically, no significant changes were observed in the kidney or liver, except for mild necrosis in the standard group. In the spleen, T1 showed expanded white pulp with increased lymphocytes, while T3 displayed apoptotic changes. Thymus tissues in the T2 and T3 groups exhibited focal epithelial hyperplasia, with T3 showing tubular epithelial formations containing eosinophilic material.

These findings demonstrate that *Loeseneriella arnottiana* bark extract, especially at moderate doses, can enhance both humoral and cell-mediated immune responses without causing major organ toxicity. The study supports the therapeutic potential of *Loeseneriella arnottiana* as a natural immunomodulator and warrants further research into its molecular mechanisms and clinical relevance.

Keywords: *Loseneriella Arnottiana*; Immunomodulatory; Povidone-Iodine; Cyclophosphamide

Abbreviations: SRBCs: Sheep Red Blood Cells; WBC: White Blood Cell; IAEC: Institutional Animal Ethics Committee; PBS: Phosphate-Buffered Saline; RBCs: Red Blood Cells; H&E: Hematoxylin and Eosin; PAS: Periodic Acid-Schiff; IHC: Immunohistochemistry; ANOVA: Analysis of Variance; SD: Standard Deviation; DTH: Delayed-Type Hypersensitivity; IgA: Immunoglobulin A; EGCG: Epigallocatechin-3-Gallate

Introduction

Loseneriella arnottiana (synonym *Hippocratea arnottiana* Wight) is a woody climbing shrub with greenish-brown bark belonging to the Hippocrateaceae (Celastraceae) family, commonly found in the Western Ghats regions of Karnataka, Tamil Nadu, Kerala and Sri Lanka. This plant has garnered attention due to its diverse therapeutic potential and is an important part of the local medicinal knowledge. The Hippocrateaceae family, which was traditionally used to classify certain plants, was later renamed Celastraceae, following taxonomic revisions that demonstrated closer morphological and genetic relationships with species in the latter family [1]. The family includes 150 shrubs and lianas. Members of this family have been widely studied for their notable medicinal properties such as antimicrobial, anti-inflammatory, anti-mutagenic, anti-plasmodial, antiviral and anti-malarial effects [2,3]. Many of these plants are used for the treatment of a variety of human ailments, owing to their rich phytochemical content. In recent years, *Loseneriella arnottiana* has gained recognition as an important medicinal plant in traditional systems of medicine, especially for the treatment of diabetes mellitus [4]. The phytochemical profile of *L. arnottiana* root reveals the presence of several bioactive compounds, including tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids [5].

Notably, methanol extracts of *L. arnottiana* exhibit the highest protein content (80%) and phenolic content (9.2%), with the highest flavonoid content being found in chloroform extracts [6]. The plant's radical scavenging activity, a key indicator of its antioxidant potential, has also been highlighted in several studies. Specifically, the methanol root extract demonstrated a radical scavenging activity of 96% at a concentration of 30 µg, comparable to the activity of 15 µg of standard ascorbic acid [7]. These findings suggest that *L. arnottiana* possesses strong antioxidant and anti-inflammatory properties, which could be explored for therapeutic use. *Loeseneriella africana*, another member of the Celastraceae family, is also known for its medicinal properties. It has traditionally been used by healers in Ghana to treat microbial infections and inflammatory conditions [8]. These studies further highlight the therapeutic value of plants, supporting their use in treating various ailments. Immunomodulatory effects of medicinal plants have gained considerable attention as they offer an alternative to conventional chemotherapy. Several studies have demonstrated the immunostimulatory properties of various plant extracts. For instance, the oral administration of petroleum ether seed extract of *Celastrus paniculatus* at a dose of 500 mg/kg has been shown to enhance both humoral and cell-mediated immunity in rats [9].

Similarly, aqueous extracts of Salacia chinensis stimulate the immune system at lower concentrations (1-4 mg/kg), although higher concentrations tend to inhibit immune response [10]. Other studies, such as the work of Zandonai, et al. [11], demonstrated the dose-dependent stimulation of T lymphocytes by extracts of *C. brasiliense*, *I. pes-caprae*, and *M. elaeagnoides*. In contrast, some plant extracts, like those of *M. robusta*, *R. imperialis*, and *V. scorpioides*, inhibited cell proliferation, indicating complex interactions between plant compounds and the immune system. In addition to immune modulation, several plant extracts have been shown to influence white blood cell (WBC) counts and lymphocyte proliferation. For example, the methanolic leaf extract of *Moringa oleifera* at a dose of 1000 mg/kg resulted in an increase in WBC, lymphocyte, and neutrophil counts in Wistar albino rats [12]. Similarly, the methanolic leaf extract of *Dendropanax morifera* increased spleen cell counts in mice [13]. These findings suggest that plants with immunomodulatory activity, such as *L. arnottiana*, can influence immune cell proliferation and contribute to overall health and disease resistance. Despite the widespread traditional use of *L. arnottiana*, there is limited pharmacological information available on this plant, which is essential for its proper authentication, quality control, and standardization.

To bridge this gap, the present study aims to evaluate the pharmacological parameters of *L. arnottiana*, providing a detailed profile that will support its accurate identification and quality assurance. Furthermore, the study will also investigate the anti-inflammatory properties of the plant, adding to the growing body of evidence for its therapeutic potential. Recent studies have shown that many compounds isolated from *L. arnottiana* exhibit strong anti-inflammatory activity, which could be pivotal in the development of novel therapeutic agents [14]. The findings will help in establishing *L. arnottiana* as a viable candidate for further pharmacological research and clinical applications.

Materials and Methods

Loeseneriella arnottiana is a shrub or woody climber or lianas (Figure 1) with simple, opposite, elliptic or ovate leaves and smooth margins (Figure 2). The plant produces small, unisexual flowers in axillary clusters, typically white or yellowish in color. Its fruit is a woody capsule containing several seeds.

Figure 1: *L. arnottiana* woody climber.

Figure 2: *L. arnottiana* vegetative habit.

Material Collection

Loeseneriella arnottiana bark was collected from the forests of Sullia, Dakshina Kannada, Karnataka. They were then washed in tap water, distilled water to remove dust and dirt. The material was chopped into small pieces which were completely dried under the shade for 20 days. Later it was finely powdered by powdering mills. The powdered sample was stored at room temperature in clean and dry container for further use.

Preparation of Bark Aqueous Extract

Soxhlet method was employed to prepare the bark extract using deionized water as solvent systems. Approximately 20g of bark sample powder was taken separately in thimble holder with 100 mL of distilled water in the Soxhlet apparatus which was run for multiple cycles until complete extraction was observed. The extracts thus obtained were concentrated using a rotary evaporator, dried and stored in airtight bottles at 4°C for further studies.

Animals and Study Design

All experimental protocols involving animals were approved by Institutional Animal Ethics Committee (IAEC) in the approval No. AAMC/CPCSEA/IAEC/2020-21 AL-03 dated 07/03/2020. Sixth and ninth week old both male and female *Rattus norvegicus* (albino strain) pathogen free were procured and acclimated to lab conditions for one week prior the work starts. The weight of rats' ranges from 150-250g. Rats were provided with standard pellet diet and *adlibitum* filtered tap water access.

Immunomodulatory Activity

Animals Grouping and Dosage

The study utilized *Albino wistar* rats, both male and female, with body weights ranging from 150-250 g. The animals were divided into 5 groups six animals in each group randomly ensuring an unbiased distribution across the groups. The plant extract was suspended in water and administered orally for 14 days. Group 1(control) received water; Group 2 (standard- Cyclophosphamide) 45mg; Group 3: T-1 (aqueous extract) 1mg/kg; Group 4: T-2 (aqueous extract) @ 2mg/kg; Group 5: T-3 (aqueous extract) @ 3mg/kg weight of the animal. For animal experiment methods of Sumalatha, et al. (2012) was followed. The dose volume was calculated not more than 1ml of drug preparation per animal. Control animals received 1 ml of water.

Housing and Environment Condition

The rats were housed individually in cages, with paddy husk used as bedding material to provide comfort and facilitate easy cleaning. The animals were maintained in a controlled environment, with a temperature of 24±2ºC to ensure optimal living conditions. Twelve hours (12 h) light/dark cycle was implemented to mimic natural day-night rhythms, supporting the rats' circadian cycles and overall well-being.

Feeding and Hydration

Throughout the study, the rats were provided with a standard pellet diet, ensuring they received adequate nutrition. Additionally, the animals had unrestricted access to water (*ad libitum*), which

helped maintain hydration levels and ensured that the experimental conditions did not introduce any additional stressors related to food or water availability. These carefully controlled housing and care conditions were implemented to maintain the well-being of the animals and to minimize any external factors that could influence the results of the immunomodulatory study.

Immunization

The experimental procedure involved the use of sheep blood, which was sourced from the Kudroli Licensed Slaughterhouse in Mangalore. The blood was collected in Alsever's solution, a commonly used preservative for blood storage. To prepare the blood for use in the study, it was washed three times with phosphate-buffered saline (PBS) to remove any contaminants or preservatives. After washing, the concentration of red blood cells (RBCs) was adjusted to 0.5×10^9 RBCs per millilitre, ensuring the appropriate concentration for the immunization procedure. On the 7th day of the study, all the rats were immunized by administering 0.5 × 109 RBCs/mL of sheep red blood cells (SRBC) intraperitoneally using an insulin syringe. The intraperitoneal injection was chosen as the route of administration to ensure efficient absorption and immune response stimulation. This method of immunization aims to induce an immune response in the rats, allowing for subsequent assessment of immunomodulatory effects. The methodology followed in this experiment was based on the protocols outlined by Bin- Hafeez, et al. [15], ensuring consistency and reliability in the experimental design. The use of SRBCs as an immunogen is a well-established technique for studying immune responses, as the introduction of foreign RBCs triggers a specific immune reaction, which can be used to evaluate the impact of various treatments or extracts on immune function.

Assessment Criteria

1. Hemagglutination Antibody Titer [15]

As much as 100 µL of serum was heat inactivated at 56°C in water bath for 30 min. About 50 µl of PBS was added to all 12 wells of microtiter plate row. First well was taken as control and was not added with serum, instead it received only PBS. Next well received 50µL of heat inactivated serum. From the same well using a micropipette, 50µL of the mixture was taken after completely mixing it with the pipette and is serially diluted by 2 fold in the subsequent wells. Finally 50 µL of SRBC with a cell density of $0.5x10^9$/mL was added to all the wells. The plate was gently tapped to mix the cells and was incubated at 37°C for 2h. The value of antibody titer was assigned to the highest serum dilution showing at least 50% of visible hemagglutination.

2. Histological Analysis of Immune Organs

Histological analysis involves several critical steps to prepare tissue samples for microscopic examination. Immune organs were carefully collected from the experimental animal and immediate fixation is necessary to preserve its cellular structures. Common fixatives like 10% neutral-buffered formalin are used to ensure proper preservation [16]. After fixation, tissues undergo processing, which includes dehydration using graded alcohol solutions (70%, 80%, 90%, 100%) followed by clearing with a solvent like xylene or toluene to prepare the tissue for embedding in paraffin wax [17]. Once embedded, tissue sections are cut into thin slices using a microtome, typically ranging from 4 to 10 microns in thickness [18]. The sections are then mounted onto glass slides and stained with various reagents such as Hematoxylin and Eosin (H&E) for general morphological examination or more specialized stains like Periodic Acid-Schiff (PAS) for carbohydrate-rich structures [19]. Immunohistochemistry (IHC) may also be employed for identifying specific proteins or markers in the tissue [20]. Once stained, the tissue sections are mounted with a coverslip using a mounting medium such as DPX, and the slides are examined under a light microscope. This comprehensive procedure allows for detailed evaluation of tissue morphology, cell distribution, and any potential pathological changes.

3. Statistical Analysis

The hemagglutination antibody titers were log-transformed ($\log_2$) prior to analysis to normalize the data distribution. One-way Analysis of Variance (ANOVA) was performed using GraphPad Prism to compare mean antibody titers across different treatment groups. Tukey's post hoc test was applied to determine significant differences between groups. A p-value less than 0.05 were considered statistically significant. For each treatment two replicates were taken. Data were expressed as mean ± standard deviation (SD).

Results

Hemagglutination Assay

The results of the hemagglutination experiment showed distinct patterns of agglutination across different test groups. In T1, no agglutination was observed in any of the wells, confirming the absence of antibody. In T3, agglutination was observed only in the first well (the undiluted serum), with no agglutination detected in subsequent wells, resulting in a titer value of 1:1. In T2, agglutination was observed in all wells, with the highest dilution showing at least 50% visible hemagglutination, corresponding to a titer value of 1:2048. This indicates a strong immune response with a high concentration of antibodies in T2. This suggests that the antibody or antigen concentration in T3 was insufficient to cause a reaction at higher dilutions, indicating a weaker immune response or lower antibody concentration compared to T2.

Statistical Analysis Results: Hemagglutination antibody titers were evaluated across five groups: Control (Water), T1, T2, T3, and Standard (Cyclophosphamide), each with two replicates. Given the exponential nature of antibody titer values, data were $\log_2$-transformed prior to analysis to normalize the distribution. One-way Analysis of Variance (ANOVA) was conducted using GraphPad Prism (v:10.2.3) to

assess differences in mean log_2-transformed antibody titers among the groups. The analysis revealed a highly significant difference between groups ($F(4,5) = 76.76$, $p < 0.001$), indicating that at least one group exhibited a statistically different immune response. Tukey's multiple comparisons test showed that the T2 group, which had the highest titer (1:2048, $log_2 = 11$), was significantly different ($p < 0.001$) from all other groups, including Control ($log_2 = -1$), Standard/Cyclophosphamide ($log_2 = -1$), T1 ($log_2 = 0.14$), and T3 ($log_2 = 0.14$). No significant differences were observed among Control, Standard, T1, and T3 groups ($p > 0.05$), indicating low or absent antibody responses in these groups (Table 1). These findings confirm that T2 induced a robust humoral immune response (Figure 3), while the remaining groups, including the immunosuppressive standard (Cyclophosphamide) and the water control, showed no or minimal antibody production.

Table 1: Antibody Response (log_2 Titer) in Different Treatment Groups.

Group	Mean ± SD (log_2 titer)	Comparison vs T2 (p-value)	Interpretation
Control (Water)	–1.00 ± 0.00	< 0.001	No antibody response
T1	0.14 ± 0.00	< 0.001	Weak antibody response
T2	11.00 ± 0.00	—	Strong immune response
T3	0.14 ± 0.00	< 0.001	Weak antibody response
Standard (Cyclophosphamide)	–1.00 ± 0.00	< 0.001	Immunosuppressed, no antibody response

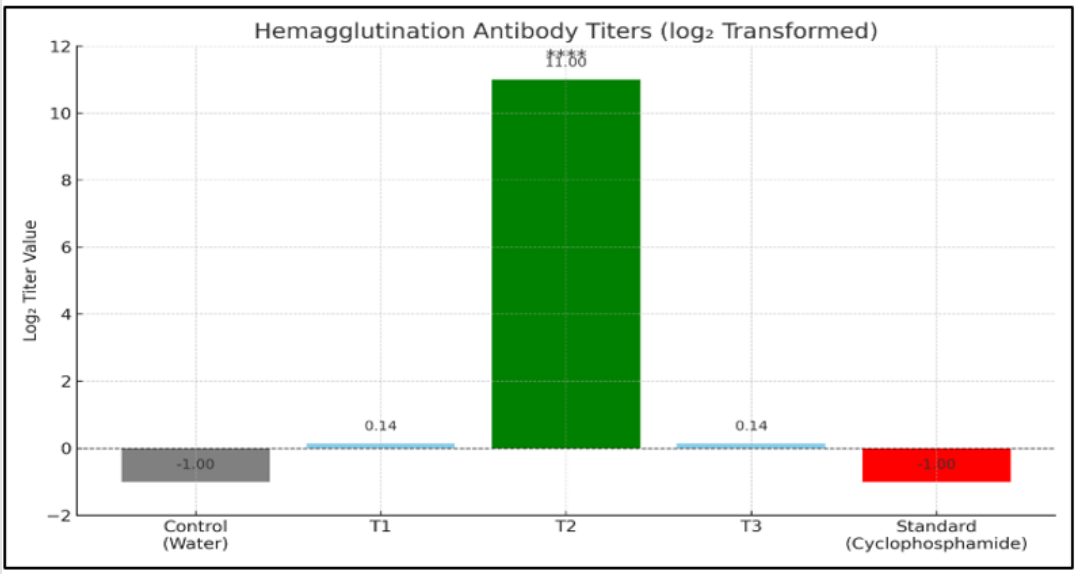

Figure 3: Hemagglutination antibody titers log_2-transformed.

Immunostimulant Related Histological Assay

Kidney

All the slides show kidney tissue consisting of cortex and medulla. The cortex consists of circular structures called corpuscles. It also contains tubules in various shapes like proximal convoluted tubule and distal convoluted tubule. Standard (Figure 4A) shows very few degenerated tubules in cortex which has pale vacuolated cytoplasm, with the cell remnants in the lumen Chronic inflammatory infiltrate seen in T3. Compared with control (Figure 4B), degenerated tubules are not seen in T1 (Figure 4C), T2 (Figure 4D), and T3 (Figure 4E).

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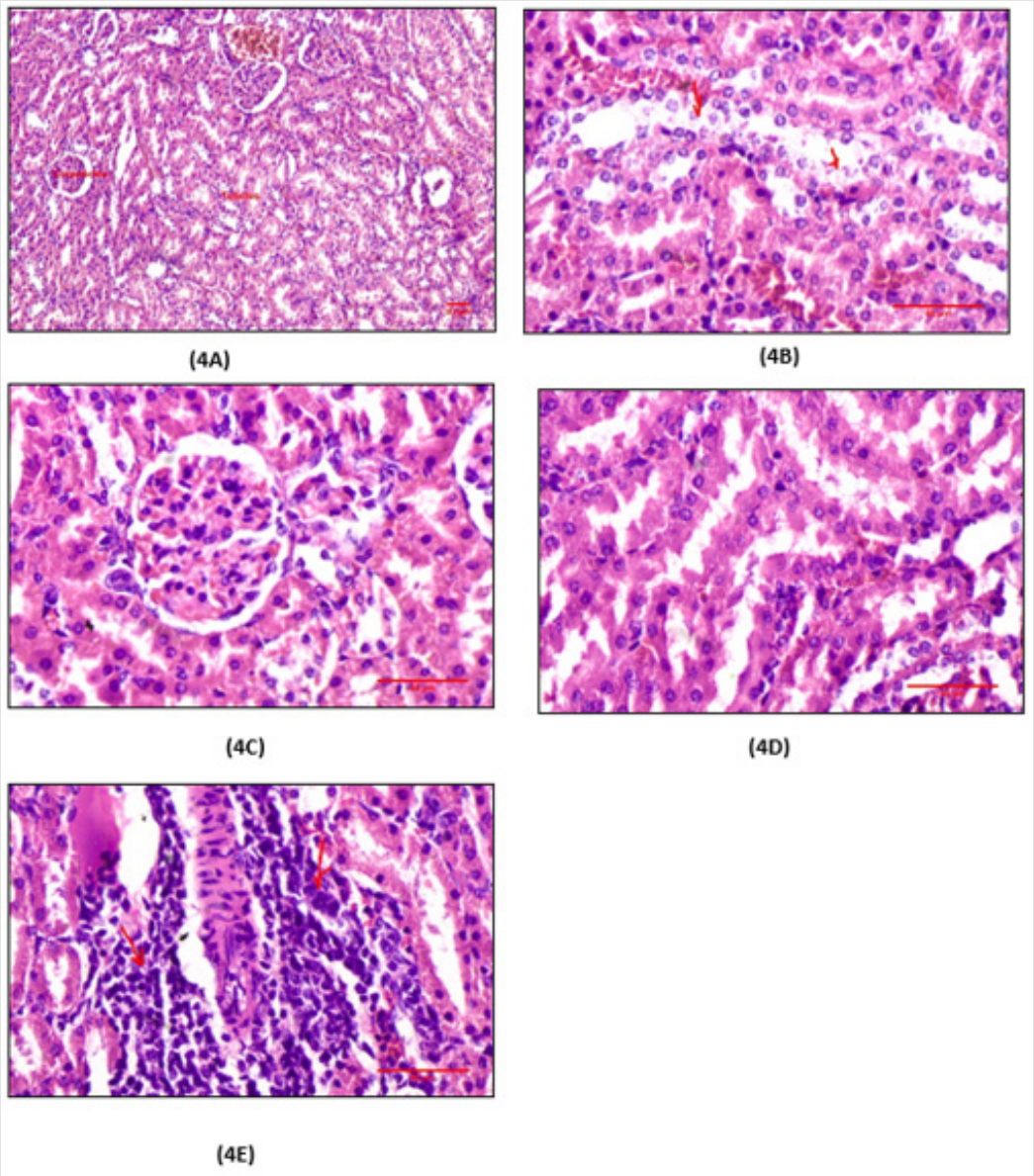

Figure 4:
A. Kidney histological assay- Control group,
B. Standard,
C. Test group 1,
D. Test group 2,
E. Test group 3.

Liver

All slides show liver tissue with lobular arrangement. Each lobule consists of a central vein and portal triads along the periphery of lobules. Numerous sinusoids pass radially from central vein and the spaces between the sinusoids contain liver cells. Compared with control (Figure 5A), standard (Figure 5B) shows necrosis in one area. T1 (Figure 5C), T2 (Figure 5D) and T3 (Figure 5E) shows no histological changes.

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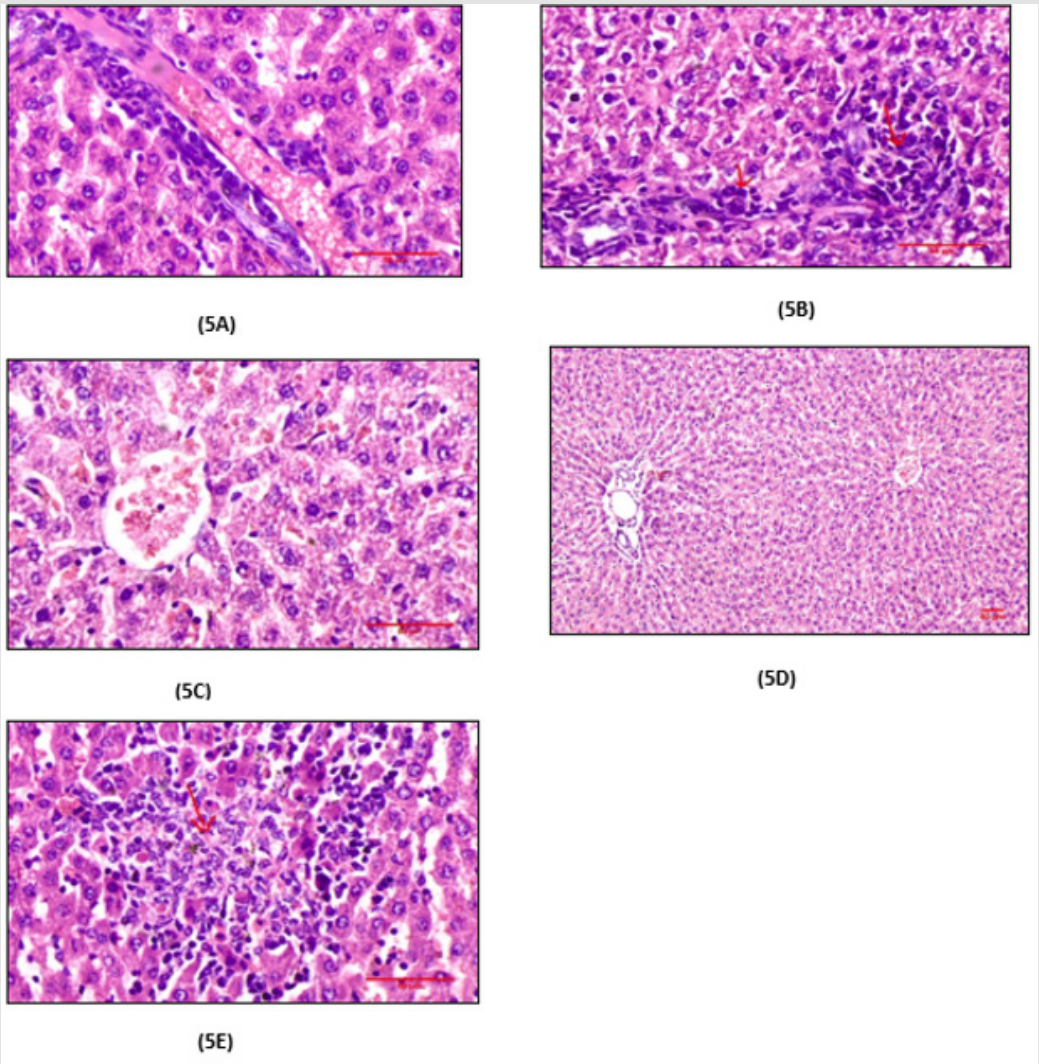

Figure 5:
A. Liver histological assay- Control group.
B. Standard,
C. Test group,
D. Test group 2,
E. Test group 3.

Spleen

All the slides show spleen tissue consisting of red pulp and white pulp. The red pulp is vascular consisting of numerous sinusoids. The white pulp consists of dense aggregations of lymphocytes in the form of cords surrounding arterioles. Standard (Figure 6A) shows slightly reduced white pulp compared with control (Figure 6B). Expanded white pulp with increase in lymphocytes seen in T1 (Figure 6C). No increase in white pulp or lymphocytes seen in T2 (Figure 6D) and T3 (Figure 6E). T3 shows apoptotic changes in lymphocytes (Tingible body macrophages).

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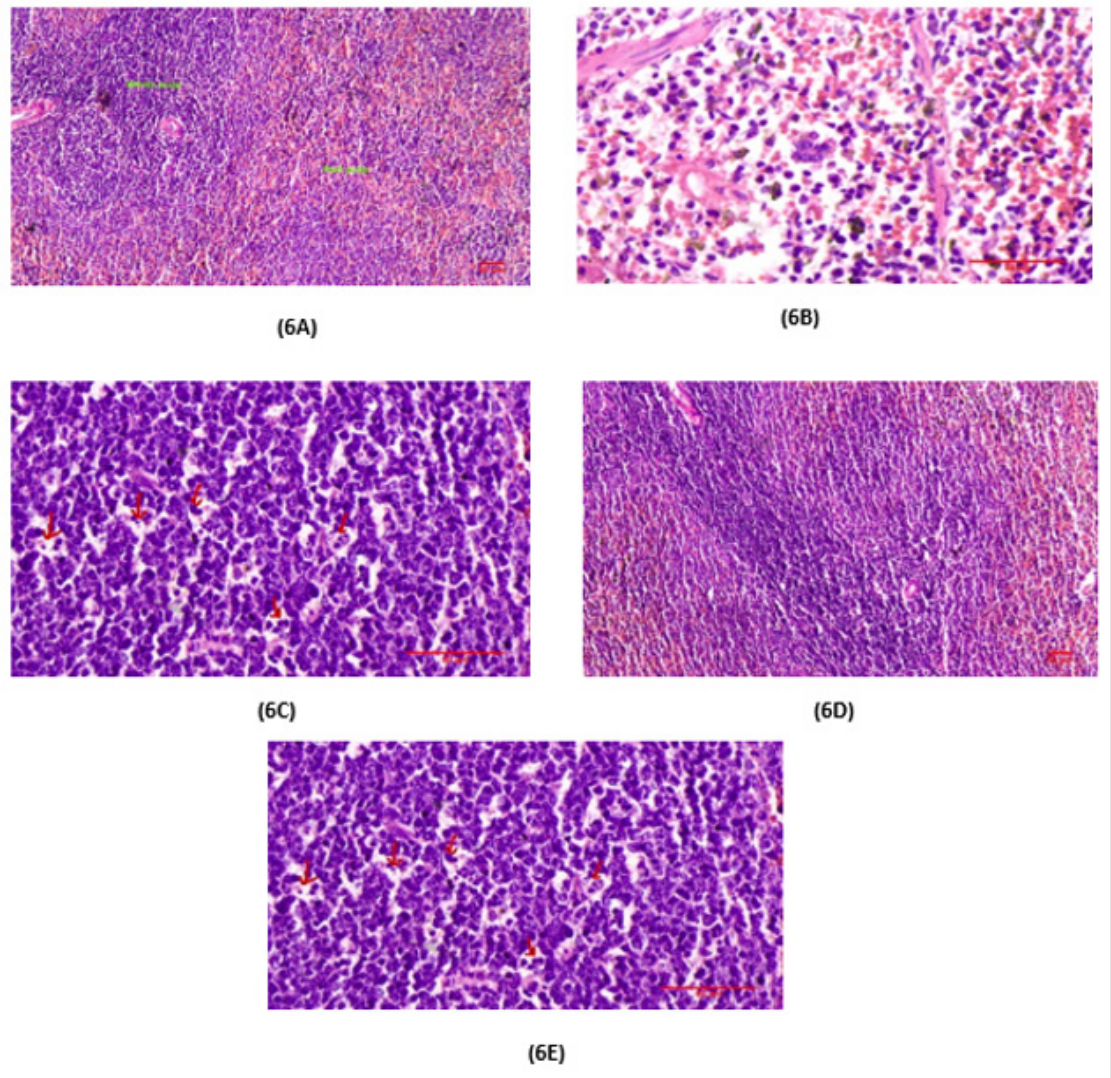

Figure 6:

A. Spleen histological assay- Control group,
B. Standard,
C. Test group 1,
D. Test group 2,
E. Test group 3.

Thymus

All the slides show thymus tissue containing cortex and medulla. The dark staining cortex consists of densely arranged lymphocytes. The pale staining medulla consists of lymphocytes and epithelial cells. Tingible body macrophages in standard (Figure 7A). No histological changes like increased or decreased cellularity, apoptotic changes are seen in control (Figure 7B) and T1 (Figure 7C) group. Focal epithelial hyperplasia (increased epithelial component in medulla) is seen in standard, T2 (Figure 7D) and T3 (Figure 7E) groups. T3 group shows tubular forms of epithelial component containing eosinophilic material. Lymph node attached to thymus shows increased apoptotic changes in lymphocytes with tingible body macrophages.

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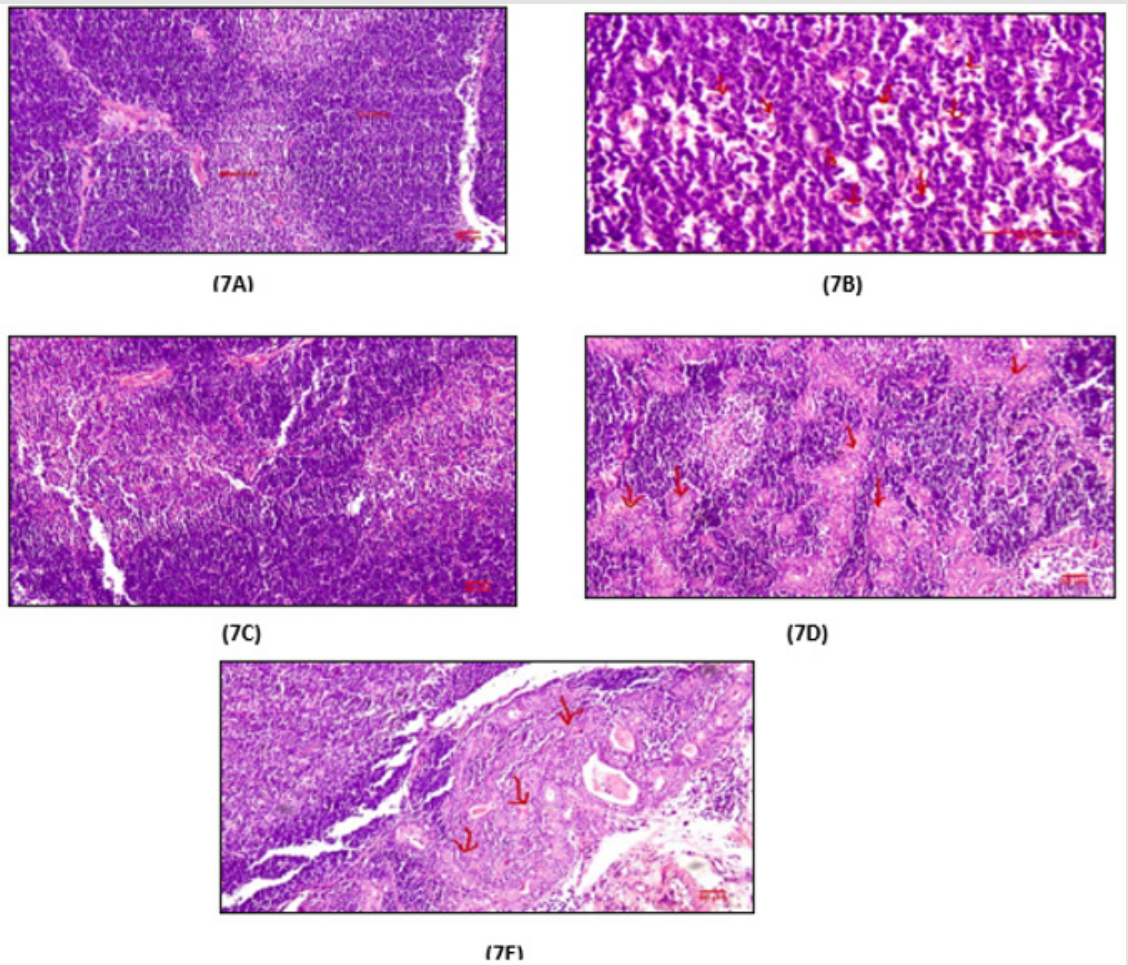

Figure 7:

A. Thymus histological assay- Control group,
B. Standard,
C. Test group 1,
D. Test group 2,
E. Test group 3.

Histological Observations Report

In the kidney, T1, T2, and T3 groups show no degeneration, while the standard group presents few degenerated tubules with chronic inflammation in T3. The liver tissues of all groups remain intact, with necrosis observed in the standard group. The spleen in T1 shows expanded white pulp with increased lymphocytes, while T2 and T3 show no significant changes, though T3 presents apoptotic lymphocytes. The thymus shows no cellular changes in the control and T1 groups, while focal epithelial hyperplasia is seen in the standard, T2, and T3 groups, with T3 displaying tubular forms of epithelial cells. Immunostimulant-related histological changes are evident in the T1 group.

Discussion

The immunomodulatory properties of *Loeseneriella arnottiana* bark extract, as revealed through the results of this study, emphasize its potential as a therapeutic agent for enhancing immune function. The outcomes from hemagglutination assays, histological analysis suggest that the bark extract may significantly modulate both humoral and cell-mediated immune responses. Furthermore, these results align with traditional medicinal uses of *L. arnottiana*, supporting the notion that the plant's bioactive compounds can stimulate immune function. The hemagglutination assay demonstrated a dose-dependent immune response to the bark extract, with the T2 group (2mg/kg) exhibiting the most substantial immune stimulation, indicated by

a high antibody titer of 1:2048. This result underscores the efficacy of moderate doses in enhancing immune response, with T2 displaying the highest immunostimulatory potential. In comparison, the T1 (1 mg/kg) and T3 (3 mg/kg) groups showed weaker responses, with T3 showing the lowest titer of 1:1. The findings are consistent with the dose-dependent immune modulation seen in other medicinal plants, such as *Celastrus paniculatus*, which was found to stimulate both humoral and cell-mediated immunity in a dose-dependent manner [9].

The immunostimulatory effects of *L. arnottiana* may be attributed to the presence of bioactive compounds in its bark extract, including flavonoids, tannins, alkaloids, and saponins. Flavonoids, for instance, are known to possess immunomodulatory properties by acting on various components of the immune system, including macrophages and T lymphocytes [21]. Similarly, tannins and alkaloids have been shown to enhance immune responses through their antioxidant and anti-inflammatory activities [6]. Thus, the plant's pharmacological potential likely stems from the synergistic action of these compounds, which work together to stimulate immune function. Histological analysis of immune organs further corroborated the immunomodulatory effects observed in the hemagglutination assay. The spleen, a central immune organ, exhibited increased lymphocyte infiltration in the T1 group, suggesting enhanced immune activity. This was not observed in the T2 and T3 groups, though T3 exhibited apoptotic lymphocytes, suggesting that higher doses may lead to lymphocyte apoptosis, potentially as a result of an overwhelming immune response or toxicity. This phenomenon echoes findings from other studies, such as that of Moringa oleifera, which showed similar dose-dependent effects on immune cell proliferation [12].

The thymus, which plays a key role in T-cell maturation, also exhibited histological changes in the T2 and T3 groups, with focal epithelial hyperplasia observed in both groups. However, T3 displayed tubular forms of epithelial cells containing eosinophilic material, which could suggest potential toxicity at higher doses. These changes highlight the importance of determining the optimal dose for therapeutic efficacy, as excessive doses might induce toxicity and hinder immune function. Notably, the liver and kidney tissues remained largely unaffected in the treatment groups, suggesting that the bark extract of *L. arnottiana* does not induce significant hepatotoxicity or nephrotoxicity at the doses tested. These findings are in line with those from other studies on medicinal plants, where minimal toxic effects were observed in vital organs, indicating the safety of the plant for therapeutic use [8]. The results of this study are consistent with previous research on other plants with immunomodulatory properties, such as Salacia chinensis, which showed that low-dose extracts could stimulate immune function, while higher doses might inhibit it [10].

Similarly, extracts from *Moringa oleifera* have been shown to enhance immune responses without causing significant toxic effects [13]. The results of these studies support the idea that medicinal plants like *L. arnottiana* could offer a natural and safe alternative for boosting immune function, particularly in individuals with compromised immune systems. The bacterial cultures showed various levels of sensitivity towards different concentration of aqueous extracts of *Cyclea peltata*. Pseudomonas aeruginosa and Streptococcus mutans showed high sensitivity against the aqueous extract of *C. peltata* (3.9 cm). Staphylococcus aureus showed low sensitivity against aqueous extract of *C. peltata* (3.6 cm) [22]. Recent investigations into other members of the Celastraceae family further support the immunomodulatory potential observed in *Loeseneriella arnottiana*. Notably, *Celastrus paniculatus*, a closely related species, has shown promising immunostimulatory effects. A study by Shah, et al. [22] demonstrated that ethanolic extracts of *C. paniculatus* seeds significantly enhanced antibody titers and lymphocyte proliferation in immunosuppressed rats, without inducing histopathological damage to the liver or kidney. These findings are consistent with the present study, where the T2 group of *L. arnottiana* exhibited strong humoral responses (titer 1:2048) alongside preserved organ integrity.

Both studies underscore the therapeutic promise of *Celastraceae* plants in immunomodulation, likely due to shared phytochemical constituents such as sesquiterpenes, alkaloids, and flavonoids, which collectively support immune system function while minimizing toxicity. The methanolic extract of *Cyclea peltata* at 200 mg/kg body weight significantly enhanced the phagocytic index (1.42) and increased total WBC count. The hemagglutination antibody titre was 128, and delayed-type hypersensitivity (DTH) response measured a footpad thickness of 0.52 mm, indicating stimulation of both humoral and cell-mediated immunity [22]. In line with these findings, current investigations into the role of gut-immune interactions and plant-based immunomodulators suggest that dietary phytochemicals can exert systemic immune effects through the gut-lymphoid axis. A recent study by Chen, et al. [23] explored how flavonoid-rich extracts from *Perilla frutescens* influenced mucosal immunity by increasing the expression of immunoglobulin A (IgA) and regulating Toll-like receptor signaling pathways in Peyer's patches. This provides a compelling parallel to the observed histological activation in lymphoid organs such as the spleen and thymus in the present study with *L. arnottiana*, indicating that similar pathways may be involved.

The mild epithelial hyperplasia and lymphocyte activation seen in T1 and T2 groups may reflect mucosal immune engagement and systemic immune priming, reinforcing the concept that orally administered plant extracts can modulate immunity via gut-associated immune mechanisms [23]. In addition to the findings presented, recent research has further elucidated the immunomodulatory properties of plant-derived compounds, particularly those found in *Camellia sinensis* (green tea). A study by Wang, et al. [24] highlighted that green tea polyphenols, especially (–)-epigallocatechin-3-gallate (EGCG), possess significant immunomodulatory effects. These compounds can enhance immune responses by modulating the activity of various immune cells, including T lymphocytes and macrophages, and by

influencing cytokine production. Such mechanisms may parallel the immunostimulatory effects observed with *L. arnottiana* bark extract, suggesting a potential role for polyphenolic compounds in mediating immune responses. Besides, Zhou, et al. [25] investigated the immunomodulatory mechanisms of tea leaf polysaccharides in mice with cyclophosphamide-induced immunosuppression.

Their findings indicated that these polysaccharides could restore immune function by modulating gut microbiota composition and enhancing the production of short-chain fatty acids, which are crucial for maintaining intestinal health and immune homeostasis. This study underscores the importance of gut-immune interactions in the context of immunomodulation and suggests that similar mechanisms might be at play with *L. arnottiana* bark extract, particularly considering the observed histological changes in immune organs [26]. Furthermore, the findings of this study contribute to the growing body of knowledge on the potential therapeutic applications of *L. arnottiana*, suggesting that the plant may be a promising candidate for the development of new immunomodulatory therapies. The dose-dependent effects observed in this study highlight the need for further research to establish the optimal dose for therapeutic use. Future studies should focus on understanding the molecular mechanisms underlying the immunomodulatory effects of *L. arnottiana* and validate its efficacy in clinical settings.

Conclusion

In conclusion, *Loeseneriella arnottiana* bark extract exhibits significant immunomodulatory activity, particularly at a moderate dose (2 mg/kg), which enhances both humoral and cell-mediated immune responses. The findings from hemagglutination assays, histological analysis, and blood component quantification suggest that the plant's bioactive compounds, including flavonoids, tannins, and alkaloids, play a key role in modulating immune function. The results also suggest that *L. arnottiana* could serve as a valuable therapeutic agent for boosting immune function, with a favorable safety profile at appropriate doses. However, further research is needed to explore the underlying molecular mechanisms, dose optimization, and long-term safety of the plant for potential use in immunological disorders. These findings are consistent with similar studies on other medicinal plants, providing additional evidence for the immunomodulatory potential of *L. arnottiana* and its promising therapeutic applications.

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