ASSESSMENT OF ANTIMICROBIAL PROPERTIES AND CYTOTOXICITY EFFECTS OF HERBAL GEL FORMULATION USING LAGENARIA SICERARIA (BOTTLE GOURD) FORTIFIED WITH HONEY FOR TREATING ORAL ULCERS- AN IN VITRO STUDY.

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Abstract

Introduction: Oral ulcers are a common, painful condition with limited effective treatment options. This study explores the potential of a novel herbal gel formulation using *Lagenaria siceraria* (Bottle gourd) fortified with honey as a treatment for oral ulcers.

Materials and methods: The gel's antimicrobial and cytotoxic activities were evaluated in vitro. *Lagenaria siceraria* was extracted and combined with honey to create the gel, which was tested against three bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using agar well diffusion and time-kill curve assays.

Results: The results demonstrated significant antimicrobial activity, with the highest inhibition observed in *P. aeruginosa*. Cytotoxicity was assessed using nauplii fish, showing minimal adverse effects at lower concentrations.

Conclusion: This study suggests that the *Lagenaria siceraria* and honey gel could be a promising, natural alternative for managing oral ulcers, combining efficacy with low cytotoxicity. Further studies are recommended to validate these findings and explore the potential for clinical application.

Key words: Lagenaria siceraria, honey, oral ulcers, herbal, oral ulcer gel.

Introduction

Oral ulcers, also known as canker sores, are a common and often recurrent condition that can significantly impact an individual's quality of life [1]. These lesions are characterized by severe pain, difficulty in eating, and a negative impact on overall oral health.

The management of oral ulcers presents a significant challenge, as many current therapies have limitations in terms of efficacy, patient compliance, and potential side effects [2, 3, 4] and this is where the need to develop an effective treatment plan comes into play. In order to do this the treatment modality needs to effectively work on the condition along with being side effect free. Herbal remedies have been used for centuries to treat a variety of medical conditions, and recent research has highlighted their potential for addressing oral health issues as well [5] Medicinal plants have played a vital role in human healthcare systems globally, not only in

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treating diseases but also in maintaining overall health and well-being [6]. These natural substances contain loads of bioactive compounds that can show antioxidant, anti-inflammatory, and antimicrobial properties [7, 8].

The need for a sustainable treatment modality that causes less side effects even on extensive use has been the need of the hour and can be a better option than the conventional commercial options, Herbal medicine has gained renewed popularity in recent years due to its cultural acceptability, affordability, and perceived safety [9].

However, the integration of traditional herbal therapies with mainstream modern medicine remains a challenge, as the scientific validation of their efficacy and safety is often lacking [10]. The current literature on the use of herbal medicines is limited by lack of evidence that supports this view. This paper will discuss the potential benefits of herbal medicine and how it can be used to treat oral ulcers.

In particular, the study will examine whether there are any significant differences between traditional and alternative methods of treatment with respect to oral ulcer prevention. The role of this study is to explore whether herbal medicine may be effective in treating oral ulcer. The present study aims to investigate whether herbal medicine may have a positive effect on the treatment of oral ulcer and to address this need in a sustainable way without side effects, the development of a novel gel formulation incorporating herbal ingredients may offer a promising solution.

The aim of the study is to evaluate antimicrobial activity and cytotoxic activity of a novel Herbal gel formulated using Lagenaria Siceraria(Bottle gourd) fortified with honey.

2. Materials and Methods:

2.1 Collection of materials

The vegetable Lagenaria siceraria was procured from a local organic store along with honey, the collection was done from a generic outlet. All the other solvents were of analytic grade.

2.2 Preparation of extracts

Lagenaria siceraria was cut and dried, then was ground for further use. 2.5 g of this Dried Lagenaria siceraria powder [Fig 1: (a)] was added to 50 ml of distilled water. The solution was then heated at 50°C for 15-20 minutes. The heated extract was filtered using number 1 whatman filter paper [Fig 1: (b)]. Then the filtered solution was concentrated up to 5 ml. Then 2.5 g of honey was weighed and mixed with the 5 ml concentrated Lagenaria siceraria solution.

2.3 Preparation of gel

10% Carboxymethyl cellulose (cmc) and 10% Carbopol 934 was mixed using a homogenizer. The extract was then added to this homogenized mixture and the gel was prepared [Fig 1: (c)].

2.4 Antimicrobial Activity

The antimicrobial activity of the Lagenaria Siceraria fortified with honey gel was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15-20 minutes. After sterilization, the medium was poured on the surface of the sterile petri plates and allowed to cool down to room temperature. The bacterial suspension (Staphylococcus aureus, Escherichia Coli, Pseudomonas Aeruginosa) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm were created in the agar plates using a polystyrene tip. The wells were then filled with different concentration (25µg, 50µg, 100µg) of the formulated Lagenaria Siceraria fortified with honey gel. Another well of an empty gel was used as a standard. Plates were incubated at 37°C for 24 hours for the culture. Antimicrobial activity was evaluated using the diameter of the inhibition zone surrounding the well. The diameter of the zone was measured using a ruler and recorded in millimetres (mm) and the zone of inhibition was calculated.

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2.5 Time-Kill Curve Assay

A time-kill curve assay was conducted to assess the bactericidal properties and concentration-dependent relation between Staphylococcus aureus, Escherichia Coli, Pseudomonas Aeruginosa over regular time intervals. The assay involved culturing the three wound pathogens in Mueller Hinton Broth supplemented with varying concentrations of silver nanoparticles (100 µg, 250 µg, 500 µg, 750 µg, and 1000 µg), followed by time-kill curve analysis. After a pre-incubation period of four hours in a medium devoid of any antimicrobial agents, growth curves were carried out before the test to ensure that all pathogens had reached a stable early-to-mid log phase. An inoculum consisting of 0.5 McFarland of each pathogen was created in sterile phosphate-buffered saline. This inoculum was collected from cultures that had been cultivated on Mueller Hinton agar plates at 37 ◦C for 18–20 h. After that, 30 µL of the inoculum was diluted in 15 mL of antimicrobial-free Mueller Hinton Broth medium that had been pre-heated to 37 ◦C, and 90 µL of the resultant mixture was distributed evenly over each well of a 96-well ELISA plate. To each well containing 90 µL of pre-incubated wound pathogens, 10 µL of the formulated Lagenaria Siceraria fortified with honey gel at 3 different concentrations along with a standard (Amoxyrite) and a control.

2.6 Cytotoxic Activity

The Nauplii fish were used for the cytotoxicity assay for the novel Lagenaria Siceraria fortified with honey gel. Firstly iodine free salt was mixed in distilled water, saline was then applied to the ELISA (enzyme-linked-immunoassay) plates. The nauplii fish were gradually lowered into the wells of the plates. After 24 hour incubation period, the formulated herbal gel in different concentrations (5, 10, 20, 40, 80 µg/mL) were added to the wells and observed.

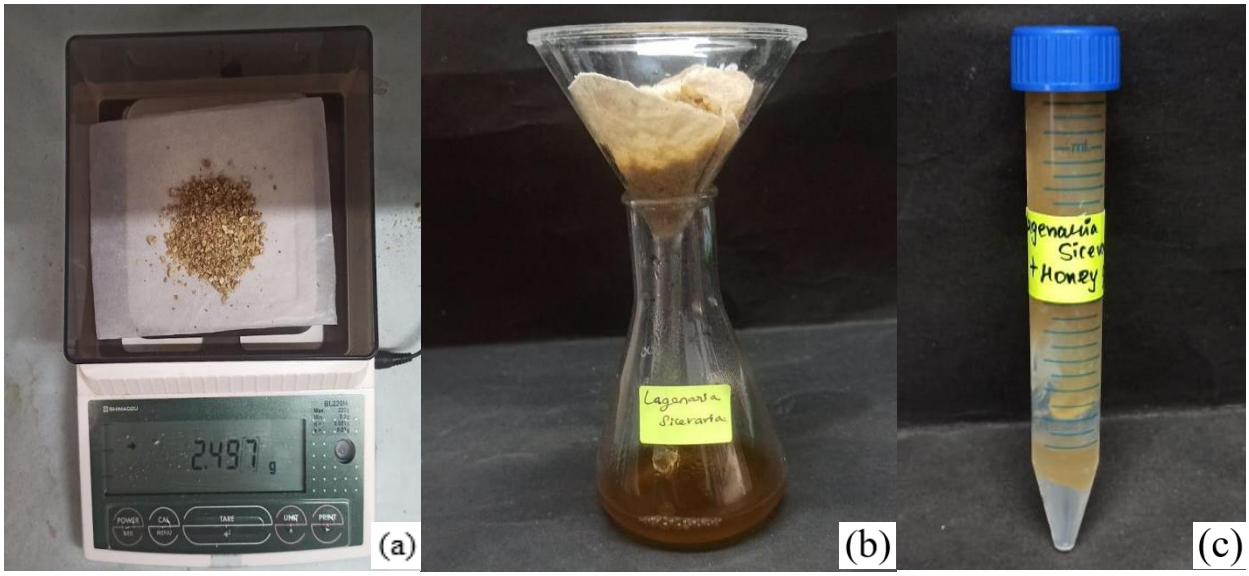


Fig 1: (a) Lagenaria Siceraria powder. (b) Lagenaria Siceraria extract. (c) Lagenaria Siceraria extract mixed with honey along with 10% Carboxymethyl cellulose (cmc) and 10% Carbopol 934

Statistical analysis

The results were expressed in count, hence only descriptive statistics were used to denote the outcome parameter at various concentration.

3. Results

The herbal gel was synthesized using Lagenaria Siceraria extract mixed with honey and was used to assess the antimicrobial and cytotoxic activity.

3.1 Antimicrobial activity

Mueller Hinton agar plates were used as medium, 3 plates were taken and

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Staphylococcus aureus, Escherichia Coli, Pseudomonas Aeruginosa separately in each plate were introduced and after incubation the herbal gel in 3 different concentrations (25µg, 50µg, 100µg) in each well of all the plates was distributed evenly, which was then observed to find out the activity. The diameter of the inhibition zone was measured using a ruler and recorded in millimetres (mm) and the zone of inhibition was calculated. The collected results are mentioned in the Table 1. The gel at 100 µg/ mL shows largest zone of inhibition in the plate containing Pseudomonas aeruginosa and least in the 25 µg/ mL concentration wells of plates containing Staphylococcus aureus, Escherichia coli [Fig 2].

Material	25 µg/mL	50 µg/mL	100 µg/mL	Control
S. aureus	9	12	15	9
E. coli	9	11	13	9
Pseudomonas Sp.	11	14	17	9

Table 1: Antimicrobial assay

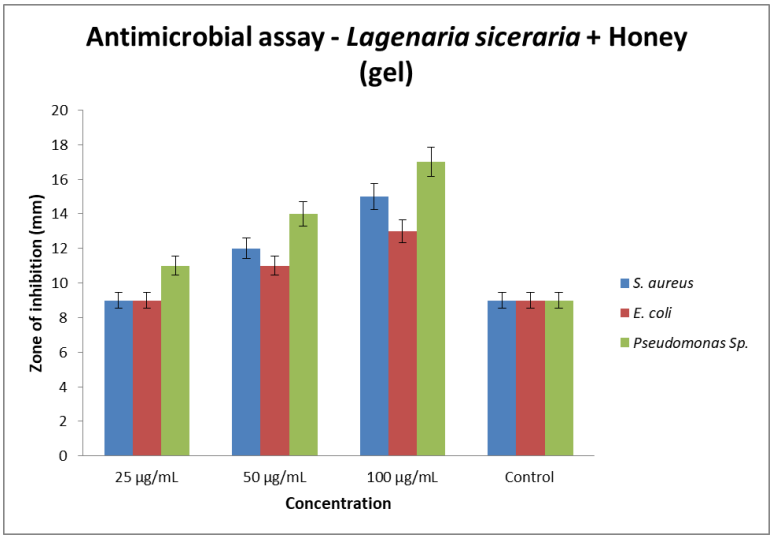


Fig 2: Antimicrobial assay comparative bar graph based on Zone diameter.

3.2 Time-Kill Curve Assay

In this study, a time-kill kinetics study was performed to investigate the antimicrobial activity of Lagenaria Siceraria fortified with honey herbal gel against three wound pathogens, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, at various concentrations ranging from 100 µg, 250 µg, 500 µg, 750 µg, and 1000 µg/L[refer Fig 3-5]. The decrease in colony-forming units, or bactericidal activity, was used to determine the efficacy of Lagenaria Siceraria fortified with honey gel in killing the bacteria. The time-kill curve assay allowed for the monitoring of the antimicrobial efficacy of Lagenaria Siceraria fortified with honey gel over time, in relation to the growth stages of the bacteria, such as the lag, exponential, and stationary phases. Our results indicate that the Lagenaria Siceraria fortified with honey gel induced a rapid and enhanced bactericidal effect against P. aeruginosa at concentrations equal to or above the

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minimum inhibitory concentration (MIC). The time-kill kinetics profile of Lagenaria Siceraria fortified with honey herbal gel against the test organisms revealed a gradual decrease in the viable cell count. .

Out of all the 100µg/mL concentration in each organism had shown highest activity and the more activity or cell death count was highest in the Escherichia coli was visible

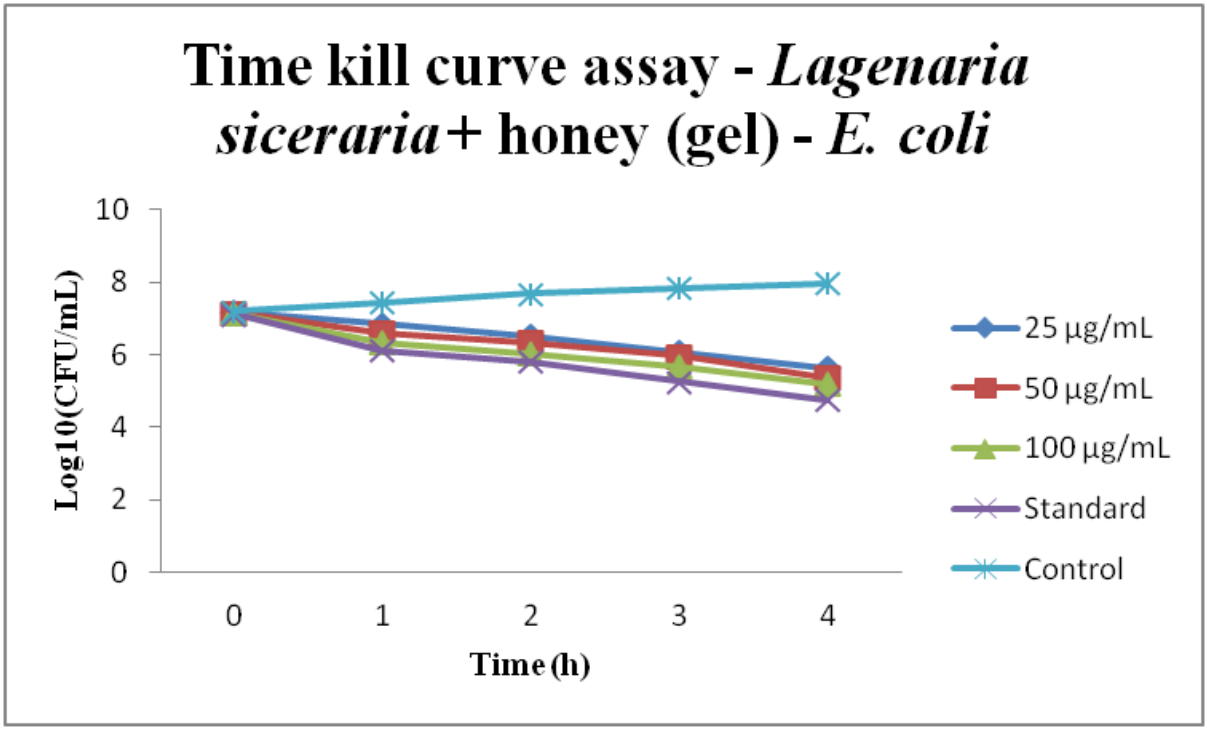


Fig 3: Time kill curve assay- Pseudomonas Aeruginosa.

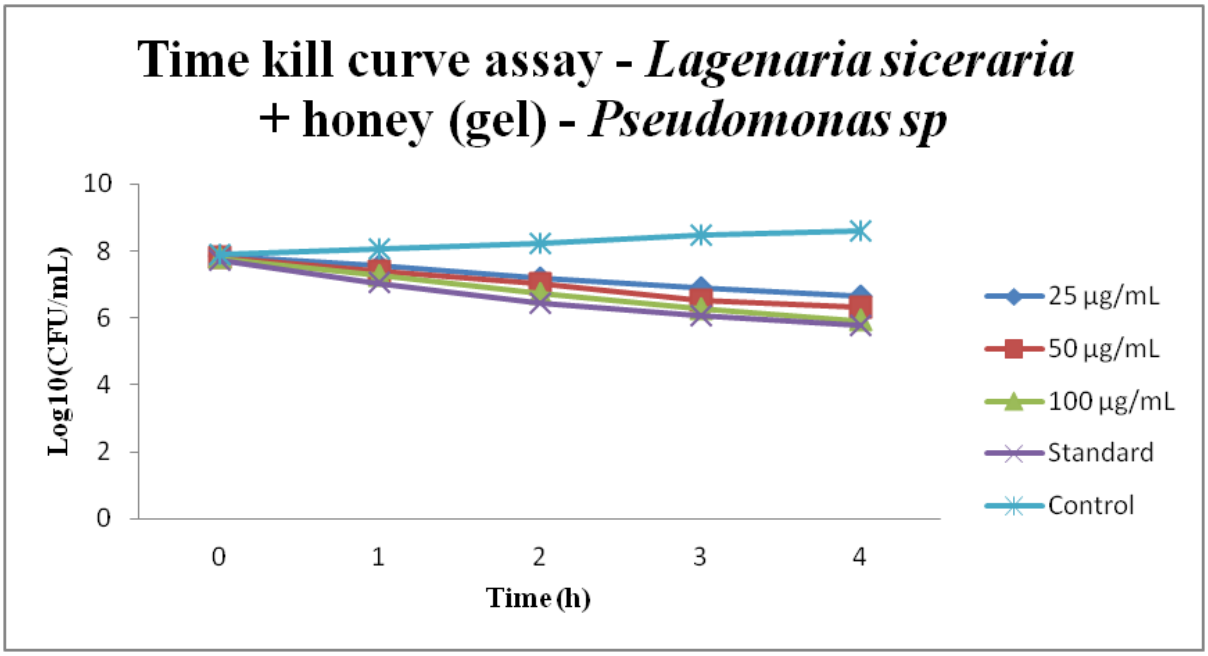


Fig 4: Time kill curve assay- Escherichia coli.

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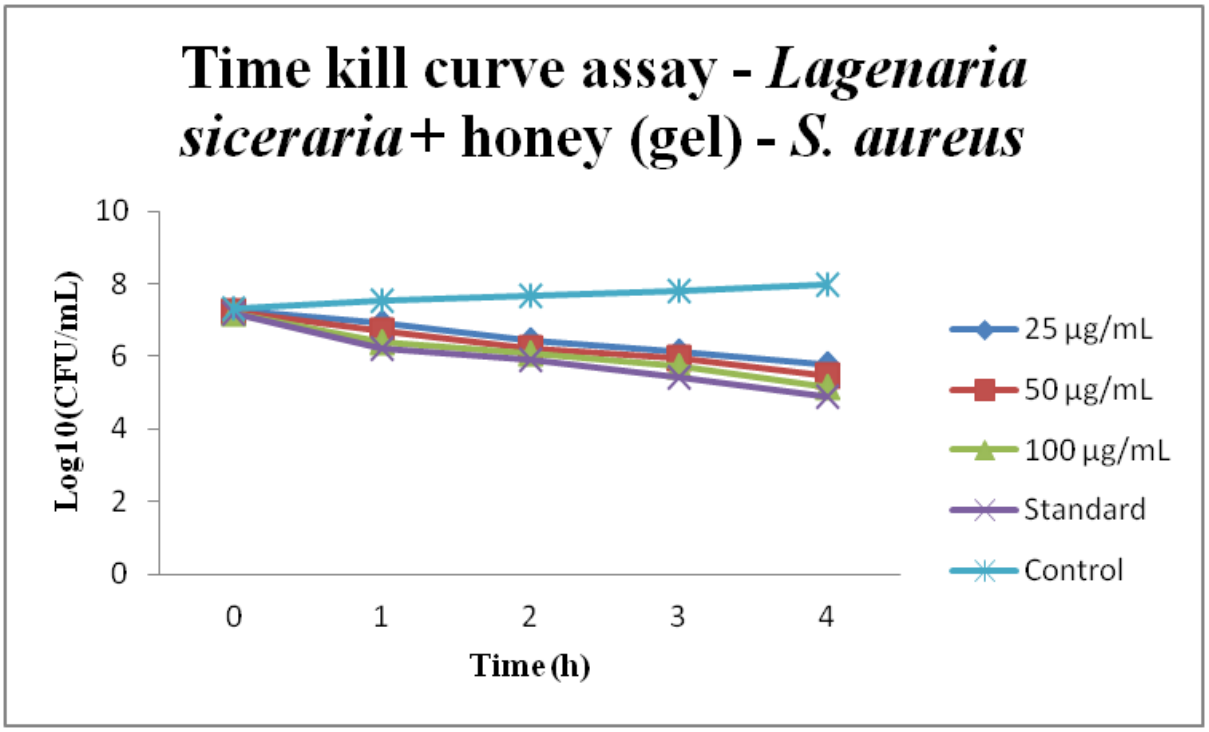


Fig 5: Time kill curve assay- Staphylococcus aureus.

3.3 Cytotoxicity activity

The experiment monitored nauplii fish response to Lagenaria Siceraria fortified with honey herbal gel over 24 hours. At 5-10µL concentration the Lagenaria Siceraria fortified with honey herbal gel sustained the nauplii fish. The fish showed no difference in percentage on the first day of all the concentrations. On day 2 while the concentration increased as to 20 µL the percentage of the nauplii fish dropped to 90% and continued to drop further with increased concentration to 40-80µL, the percentage of nauplii fish dropped to 80%. This proves the preservation of majority of the nauplii fish at higher concentrations [Fig 6].

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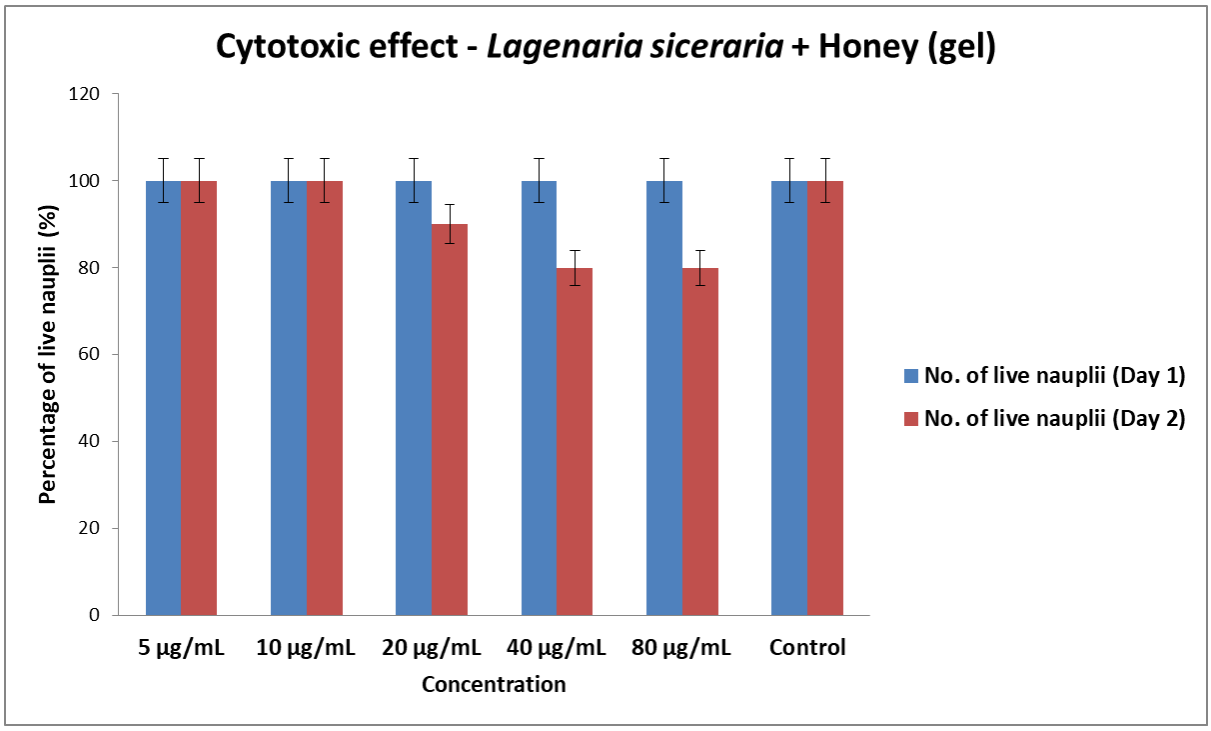


Fig 6: Cytotoxic effects of Lagenaria Siceraria fortified with honey herbal gel.

Discussion

Prajapathi RP et al, states that Lagenaria siceraria is a significant plant in Indian Systems of Medicine (ISM) and folk medicine, particularly for cardiac and hepatic diseases, ulcers, and more. Its fruit is widely cultivated in India, Sri Lanka, China, and other countries for culinary and medicinal purposes. The plant contains various bioactive compounds, including flavonoids, saponins, and triterpenes, which contribute to its therapeutic properties. Scientific studies support its use in treating multiple ailments, making it an important contribution from Ayurveda to health and wellness [11].

Thube et al, states that herbal extracts have properties and activity that can improve wound healing and prevent from oxidative damage and that wounds treated with individual gel formulations and their polyherbal formulation extracts significantly accelerate the wound healing process. This wound healing process attributed probably due to the tannins can be considered as a very important factor in reducing ulceration [12].

Ahmed SR et al, states that all around the world, there are huge numbers of medicinal plants but the medicinal value of less than one-third of them have been discovered and identified. Still now, more comprehensive, and reliable scientific studies are needed to evaluate and ensure the safety and efficiency of medicinal plants, as well as their potential metabolites in ulcers and inflammatory disease treatment. The quality of the targeted active compounds should be tested repeatedly or batch-to-batch for ensuring reproducibility as well as uniformity of the active components [13].

Srivastava et al, state that methanol caused a significant decrease in the gastric volume, free acidity, and total acidity compared to the control group, indicating an antisecretory mechanism. Several scientific studies revealed that phytoconstituents like flavonoids, tannins, terpenoids, and saponin were responsible for

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gastroprotective agents. These were extracted from Lagenaria siceraria [14].

Zahoor et al, states that this vegetable has low caloric values and high water contents. The edible portion of it contains phytochemicals like vitamins, proteins, choline, minerals, terpenoids, flavonoids, etc. Several bioactive compounds have been isolated from L. siceraria, including triterpenoids, sterols, cucurbitacins, flavones, C-glycosides and β-glycosides. Researchers have evaluated various parts of this plant viz. fruit, root, flowers, and leaves for pharmacological activities like antianxiety, antidepressant, diuretic, antimicrobial, cytotoxic, antihyperlipidemic, cardio protective, analgesic, anti-inflammatory, anthelmintic, anti-hyperglycemic, antihepatotoxic, anti-urolithiatic, antistress, antiulcer, anticancer, hepatoprotective, anthelmintic, immunomodulatory, and antioxidant. All the results in the study state that the use of herbal ingredients is the way to sustainable and side effect free treatment modalities [15].

Conclusion: Based on the results of the present study, *Lagenaria siceraria* and honey gel could be a effective indigenous alternative for the management of oral ulcers due to its less cytotoxic effect. Further clinical trials need to be conducted to evaluate its beneficial effects.

Clinical significance: Recently, Herbal therapeutics plays a pivotal role in the prevention, diagnosis, and treatment of a disease or to modify a physiological process [16] and also, there has been more focus towards the use of natural, chemical free herbs[17],homeo-based[18] eco-friendly products [19] possessing anti-inflammatory and antimicrobial properties[20] for the treatment of oral diseases. Therefore, , *Lagenaria siceraria* and honey gel which are plant based products can be a suitable and safe alternatives for the treatment of oral ulcers.

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