ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 09 | September - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSESSMENT OF THE ANTIBACTERIAL AND CYTOTOXICITY PROPERTY OF COMBINATION GEL CONTAINING ALOE VERA AND NEEM EXTRACT.

Dentistry

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ABSTRACT

The use of medicinal plants in the treatment of various ailments in dentistry is gaining popularity because of their therapeutic uses with fewer side effects. Among these herbal agents, Aloe vera and Neem have various pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic properties. Hence the study aimed to evaluate and compare the antibacterial efficacy and cytotoxicity of Combination gel containing Aloe vera and Neem against Porphyromonas gingivalis (P.g) and Aggregatibacter actinomycetemcomitans (A.a). The combination gel of aloe vera and neem was prepared using ethanolic extract of Aloe vera and Neem. Its MIC and MBC was assessed against (P.g) and (A.a). L929 cell lines were used for the MTT assay for determining the cytotoxicity of the prepared combination gel. The mean MIC and MBC value of combination gel against (P.g) was 1.25 and 2.5 respectively. The mean MIC and MBC value of combination gel against (A.a) was 1.25 and 2.5. By MTT assay at all concentrations 50%, 25%, 12.5%, 6.5%, 3.12% of combination gel, L929 fibroblasts cells showed no cytotoxicity. Thus, it can be concluded that the Combination gel containing Aloe and neem showed antibacterial effect and showed no cytotoxicity towards the L929 cell lines. Since the fibroblasts were viable at all concentrations of the prepared gel.

KEYWORDS

Aloe vera gel, Chronic periodontitis, Combination gel, MTT assay, Neem gel.

INTRODUCTION

The primary objectives of Periodontics is to preserve the health and integrity of the periodontium. Periodontitis is the most common widespread health problem because of its high prevalence rate of 25% to 55% worldwide, leading to tooth loss and deteriorating the standard of life.[1] Inflammation and destruction of periodontal tissues are considered to result from the response of a susceptible host to a microbial biofilm containing gram negative pathogens.[2]

The most common bacteria associated with periodontitis are P.gingivalis, A.actinomycetemcomitans, F.nucleatum, T.forsythia, P. intermedia, C.rectus, E.corrodens. Among these P. gingivalis and A. actinomycetemcomitans are known to invade host tissues and their association has been strongly incriminated in destructive form of periodontitis.[3]

Scaling and root planing, involves mechanical debridement of plaque and calculus and is considered the "gold standard". But it often has reduced effectiveness in the subgingival environment.[4]

Locally delivered anti-infective pharmacological agents in the form of sustained-release delivery systems have numerous clinical, pharmacologic and toxicologic advantages over conventional treatments for periodontal diseases. Local application directly to the pocket can facilitate the administration of a drug that cannot be given orally.[5]

Various plant extracts, co-enzymes and vitamins have been used to enhance the firmness and stability of the tissues maintaining the integrity of oral cavity. They are favoured for being cost-effective, relatively safe and associated with reduced development of resistance, toxicity and fewer side effects, including hypersensitivity reactions and staining of teeth, compared to conventional antimicrobial agents.[6]

Aloe Vera and Neem, two popular traditional Indian medicinal plants that have been used since the early 1700s. contain natural phytochemicals which have been considered useful alternatives to synthetic drugs and have been used therapeutically for a long time to treat many diseases. Aloe vera (Aloe barbadensis miller), and their derivatives like emodin, anthrone, Isobarbaloin and chromones in Aloe Vera leaves exert a strong purgative effect and possess potent anti-microbial agents.[7] Neem (Azadirachta indica), is one such plant which belongs to family Meliaceae, is the most versatile, multifarious trees of tropics, with immense potential. Neem possesses antibacterial, antifungal, antiviral, antipyretic, anti-inflammatory, antimalarial, antitumor, and diuretic effects.[8]

Hence the study aimed to assess the antibacterial and cytotoxic property of combination gel containing Aloe vera and neem extract.

MATERIALS AND METHOD

Study design.

The study was conducted at the KAHER's Dr. Prabhakar Kore's Basic Science Research Centre (BSRC), Belagavi, Karnataka. The gel preparation was done in KAHER's KLE College of Pharmacy, Belagavi.

1) Method Of Preparation Of The Ethanolic Extracts:

The fresh leaves of Aloe vera and neem were collected and stored in airtight containers. The leaves were dried at 70ºC for 2 hours and then powdered. Approximately 200g of Neem powder was soaked in 1600ml of 90% ethanol and 50g of Aloe vera powder in 200ml of 90% ethanol for 72 hours at room temperature. The extracts were filtered using Whatman No.1 filter paper. Evaporation of the filtrate was done using the "New Brunswick scientific Excella E24 Incubator Shaker Series" at 40ºC until it was concentrated. The extract was sterilized overnight by UV irradiation and was stored at 4ºC.

2) Method Of Inoculum Preparation: BHI broth and ATCC strain of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans were used for the preparation of inoculum. A sterile loop was used to pick the colonies, and was transferred into a tube containing 5 mL of BHI broth. This stock culture was incubated at 37°C for 8–14 hours until it attained the turbidity of the 0.5 McFarland standard.

3) Broth Dilution Method [resazurin] For Determining Minimum Inhibitory Concentration (mic): The MIC of the extract was assessed in various combination of Aloe vera and neem in 1:1, 1:2 and 2:1 ratio respectively. Broth dilution was done in a sterilized 96-well plate. In the first well 100 µl of the extract was added and serially diluted to the required concentrations up to the eighth well. A similar procedure was performed in the other two rows of the well plates and was incubated in a McIntosh and Fildes' anaerobic jar followed by the addition of resazurin reagent after 48 hours. The possible colour change from blue/violet to slight pink /pink / magenta was recorded after 4 hours as MIC of emulsion. The best result for MIC was obtained in the 1:2 ratio of Aloe and Neem which was then used for gel preparation

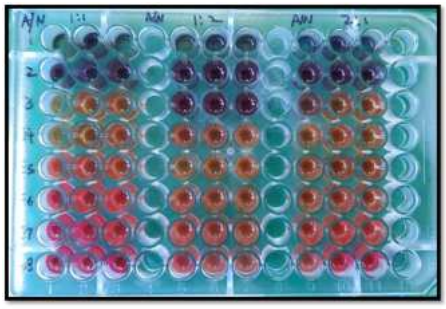

Figure.1 Minimum Inhibitory Concentration of Aloe vera and Neem at 1:1, 1:2 and 2:1 ratio respectively.

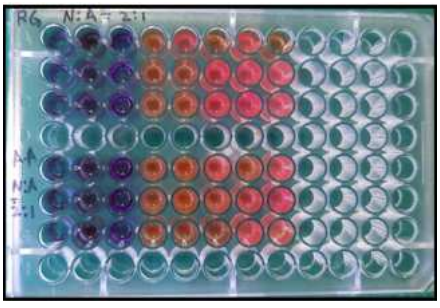

Figure.2 MIC of Neem and Aloe gel (2:1) ratio against (P.g) and (A.a).

4) Minimum Bactericidal Concentration (mbc): MBC was checked using the MIC values of both Aloe vera and neem extracts. With the help of inoculating loop, streaks were made on the BHI agar plates. The plates were sealed with the paraffin film and were incubated in the bacteriological incubator for 12 hours. At the end, minimum concentration at which the bacteria did not show any growth was considered as MBC value. (Table 1)

Table 1: MIC and MBC results of Combination gel.

Extract Name	Porphyromonas gingivalis				Aggregatibacter actinomycetemcomitans			
	MIC		MBC		MIC		MBC	
Combination gel	1.25		2.5		1.25		2.5	
	1.25	1.25	2.5	2.5	1.25	1.25	2.5	2.5
	1.25		2.5		1.25		2.5	

Figure.3 MBC of Combination gel against (A.a) and (P.g).

5) Procedure For The Preparation Of Combination Gel:
The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Aloe and Neem extract was used to prepare the combination gel.

Preparation of Carbopol 940 gel base:

a) Weighed quantity of 1% Carbopol 940 was added to distilled water gradually to prevent clumping and to promote uniform distribution.
b) Then, it was stirred continuously with a magnetic stirrer for three hours and kept 24 hours for complete hydration.

Preparation of Extract Dispersion:

a) Neem and Aloe vera extracts (2:1 ratio) was triturated in a mortar and pestle.
b) Tween 80 and Propylene glycol was added to the triturated extract and uniformly dispersed.
c) Distilled water was added to the above triturated extract along with preservatives like sodium methyl paraben, sodium propyl paraben and sodium benzoate. The solution was then stirred with a magnetic stirrer for 30 mins at 700 rpm.

Gel Formation:

d) The extract dispersion was added to the Carbopol 940 gel base and the volume was adjusted with distilled water to achieve the final weight of 100 gm of gel.
e) Triethanolamine was added dropwise to the above mixture and stirred using magnetic stirrer at 1200rpm for 30 mins.
f) The gel was then passed through UV irradiation for 20-30 minutes.
g) Then was transferred into an airtight container and stored appropriately for future use.

Figure.4 Prepared Combination gel of Neem and Aloe vera (2:1 ratio).

Table 2: Composition of Combination gel containing Aloe vera 2% w/w and Neem 4% w/w

SL No.	Ingredients	Formulation	Function
1.	Aloe vera	2% w/w	Natural active ingredient
2.	Neem	4% w/w	Natural active ingredient
3.	Carbopol 940	1% w/w	Gelling agent
4.	Tween 80	0.06% w/w	Dispersing agent
5.	Propylene glycol	2% w/w	Plasticizer and Humectant
6.	Sodium methyl paraben	0.033% w/w	Bactericidal agent
7.	Sodium propyl paraben	0.066% w/w	Bactericidal agent
8.	Sodium benzoate	0.03% w/w	Bacteriostatic agent
9.	Triethanolamine	0.5% w/w	pH adjuster and stabilizer
10.	Distilled water	q.s	Solvent

6) Method To Check Cytotoxicity Of The Combination Gel Maintenance Of Cell Lines: Cell lines of L929 was procured from National Centre of cell Sciences (NCCS) Pune. Maintenance and subculturing of the cells was done by preparing 100ml of complete media compromising of DMEM (89ml) (Hi media, Ref: AL250A) FBS (10ml) (Hi media and antibiotics) (1ml). The cells were maintained in 5% CO2 incubator in Class 2 cabinet at all aseptic conditions.

Method Of Mtt Assay: During the cells in log phase of growth MTT assay was performed. 96 well plates marking was done by considering negative control (without adding gel), combination gel of Aloe vera and Neem of 50%, 25%, 12.5%, 6.25%, 3.12% concentrations in triplicate wells. 10,000 cells/well were seeded in each well. After 24 hours, 20 micro liters of MTT dye was added and incubated for four hours. The supernatant was slowly removed and discarded without disturbing the formazan crystals, 100 micro liters of DMSO was added to dissolve the crystals and by using Spectrophotometer at around 570nm reading was obtained and calculated the proliferative index by dividing the OD of test with OD of control multiplied by 100.

Table 3: Cell Proliferation index of MTT Assay

Combination gel	Proliferation index
Negative control	100%
50%	180%
25%	201%
12.5%	135%
6.25%	128%
3.12%	134%

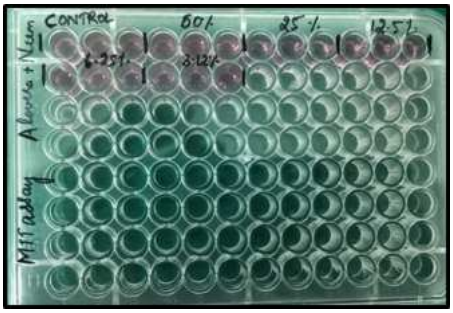

Figure.5 Cytotoxicity: MTT Assay

RESULTS AND OBSERVATION

Its MIC values were 1.25 against P.g and A.a and MBC values were 2.5 against P.g and A.a**.**

Aloe vera and neem gel was able to maintain the viability of L929 fibroblasts at all concentration of 50%, 25%, 6.25%, 3.12% of combination gel through MTT assay, thus proved to be cytoprotective in nature.

DISCUSSION

Periodontal disease is initiated by microbial plaque. Various mechanical methods and chemical agents are used to prevent the formation and deposition of microbial plaque.[9]The therapeutic goal in LDD is achieved by placing the antimicrobial agent directly in the periodontal pocket, which releases the active drug in a controlled fashion to combat the microbial attack, simultaneously minimizing its undesirable systemic effects. [10]

Most chemotherapeutic drugs have lost their therapeutic effect due to drug resistance by microorganism. Therefore, in the present scenario, there is a dire need to develop herbal preparations with minimal effect on the normal cells. [11] Aloe vera and neem are among such herbal agents and possess anti-bacterial, anti-viral, anti-fungal, anti-inflammatory and antioxidant. [12]

In the present study, MIC of Aloe vera and Neem gel against P.g and A.a was observed at 1.25 mg (**Table 1**). The MBC of the Aloe vera and Neem gel for P.g and A.a was 2.5 mg (**Table 1**). These results show that the active constituents of both Aloe vera and Neem show significant anti-bacterial activity.

In vitro cytotoxicity screening assays provide a measure of cell death caused by materials or their extracts. The effect a material may have on cell survival, is an apparent determinant of biocompatibility. Therapeutic materials during their clinical use should have desirable action, maintain maximal tissue vitality and at the same time should have negligible or no cytotoxic effects. [13]

MTT assay 3-(4,5-dimethylthiazol-2yl)2,5-diphenyltetrazolium bromide is a quantitative test with which a linear relationship between cell activity, absorbance and hence, the cell growth or cell death rate can be measured.[14]

In our study it was found that at all concentrations 50%, 25%, 12.5%, 6.25%, 3.12% of combinations gel showed no cytotoxicity on L929 fibroblasts cells. Higher concentration of gel showed increase in proliferation index of L929 cells than compared to lesser concentration of gel.

Curto et al 2014 stated that Aloe vera is nontoxic and can increase the proliferation of fibroblasts and improve cell viability. Gontijo et al 2013 concluded a greater concentration of Aloe vera extract is needed to maintain or increase cell viability. [15]

Study done by Verma et al 2018, revealed that cells were more sharply distinct in all 3 phases of cell cycle with 1% Neem extract. As its concentration is raised from 25% to 100%, the Propidium Iodide uptake does not alter much too adverse status at 50% and 100% concentration, respectively. (Umesh Verma et al. 2018). [16]

Thus the in vitro studies conducted would greatly help extrapolate the situation under in vivo conditions that exist in a human where fibroblast interaction with other cellular components including the vasculature is crucial. Hence, further in vivo study needs to be undertaken.

CONCLUSION

Combination gel can be used as a component in local drug delivery system due to its antibacterial activity and cytoprotective nature. Thus, further clinical trials are required to assess the clinical parameters on patients with chronic periodontitis.

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