Research Article

Comparative Evaluation of Biocompatibility of *Madhuca Longifolia* Saponin Seed Extract with Sodium Hypochlorite-*In Vitro* Study

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Abstract

Background: Success of a root canal therapy depends on how well a clinician eradicates the microorganisms from the complex root canal system. Synthetic irrigants have been associated with harmful side effects. Herbal products with minimal side effects are gaining importance. **Objectives:** To evaluate the biocompatibility of novel irrigant from *Madhuca longifolia* seed extract on the L929 cell lines. **Methods:** The extract was prepared by using 10 gm of the powdered sample of *Madhuca longifolia* with 85% methanol using Soxhlet extraction method. L929 fibroblast cell lines were purchased and cells were cultured in a humidified atmosphere at 37°C in the cell growth Dulbecco's modified eagle medium. Cytotoxicity test was performed using MTT assay. The median lethal concentration of the test samples were expressed as the percentage survival of the cells. One-way analysis of variance (ANOVA) and post hoc least-significant difference test was done. **Results:** Saponin extract showed 75% cell viability but Sodium hypochlorite showed a cell viability of 22%. *Madhuca longifolia* saponin seed extract at 300 and 400 micrograms concentration had a higher percentage of cell viability than the positive control, Sodium hypochlorite. Results were statistically significant with P value less than 0.05. **Conclusion:** *Madhuca longifolia* seed extract has been proven to be more biocompatible than sodium hypochlorite. It is also proven to have good antibacterial properties. *Madhuca longifolia* has the potential to be used as an intracanal irrigant.

Keywords: Biocompatibility; *Madhuca longifolia*; Sodium hypochlorite; L929 cell lines; Intracanal irrigant

Introduction

Success of a root canal therapy depends on how well a clinician eradicates the microorganisms from the complex root canal system.

The root canal system is complex and accessory features, such as fins, cul de sacs, and inter canal communications, are colonized by microorganisms once the tooth becomes infected. [1-15]

The role of microorganisms in the development and progression of pulp and periapical diseases has clearly been demonstrated. [16] Persistence of microorganisms increases the chances of failure.

Many protocols have been described to reduce the microbial load in the root canal system like various instrumentation techniques, irrigation regimens and intracanal medicaments [17-26].

It is said that mechanical instrumentation gives the shape and widens the canal but the disinfection is achieved only by means of irrigants and intracanal medicaments.

There is a general belief that mechanical enlargement of canals with simultaneous irrigation facilitates maximum removal of microorganisms. [27-33]

So, the use of chemical agents during instrumentation to completely clean all aspects of the root canal system is central to successful endodontic treatment.

Irrigation dynamics are the factors which are responsible for bringing the irrigant in contact with the microorganisms and

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containing 5% CO_2 and 95% air for 24 h to permit attachment of the cells to the plates.

Cytotoxicity test by MTT assay

The EMEM, Kanamycin, 1% penstrep, 10% FBS, 100 units/ml of fungizone 100 µl was added as a control media to the 96 well microtiter plates. The microplates are filled with 100 µl of fibroblast cells with a density of 3×10^3 in EMEM, kanamycin, 1% penstrep, 10% FBS, 100 units/ml of fungizone as negative control. The cells were allowed to adhere for 24 hours, and the growth medium using a micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS [Figure 4].

Figure 4: MTT Assay in microtiter plate.

1 ml of medium (without FBS) containing different dilution of seed extract (300 and 400 µg/ml) were added in respective wells; 20 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells incubated for a further 6-7 hrs in 5% CO_2 incubator. After removal of the medium, 1 ml of DMSO was added to each well and the positive control (Sodium hypochlorite) was tested. The supernatant was removed. 50 µl of propanol was added to plates. Plates were gently shaken to solubilize the formed formazan and the absorbance was read on an Enzyme-Linked Immunosorbent Assay (ELISA) reader at 570 nm. Each experiment was carried out in triplicate and the LC50 of the test samples as the percentage survival of the cells was calculated [Figure 5].

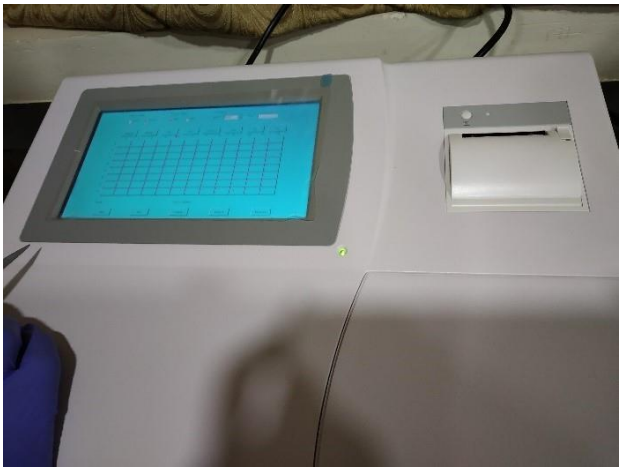

Figure 5: Absorbance rate was measured using ELISA reader.

Results

Madhuca longifolia seed extract had a higher absorbance rate than Sodium hypochlorite. Saponin showed at 300 and 400 ug concentration had 75% and 60% cell viability. Sodium hypochlorite at 3% had shown 22.7% cell viability [Table 1, Table 2], [Graph 1, Graph 2].

Table 1. Showing the percentage of cell viability for control sample (Sodium hypochlorite) and test sample (*Madhuca longifolia* seed extract 300 ug and 400 ug concentration). *Madhuca* seed extract showed 74.5% and 60.5% of cell viability at 300 and 400 micrograms respectively. *Madhuca longifolia* saponin seed extract at higher concentrations had a higher percentage of cell viability than the positive control, Sodium hypochlorite. Results were statistically significant with P value less than 0.05.

S. No.	Treatment	Concentration	Percentage of cell viability
1	L929 untreated cells	-	100
2	Saponin	300	74.5
3	Saponin	400	60.5
4	NaOcl	3	22.7

Table 2. Showing the absorbance rate of L929 cells after treatment with control and test sample. *Madhuca longifolia* saponin seed extract at 300 and 400 micrograms showed higher absorbance rate than Sodium hypochlorite. Results were statistically significant with P value less than 0.05.

S. No	Treatment	Concentration	n	Absorbance rate
1	L929 untreated cells	-	1	0.516
			2	0.489
			3	0.552
2	*Madhuca longifolia* saponin seed extract	300	1	0.396
			2	0.369

the crude ethanolic extract, saponin mixture and methanolic bark extract of *Madhuca longifolia* extract has shown significant effect on inflammation induced by carrageenan. [65,66] It was proposed that *Madhuca longifolia* inhibits the prostaglandin synthesis and also acts by reducing the intercellular cell adhesion molecule-1 which in turn is induced by tumor necrosis factor alpha. [67] It is also a good analgesic. Chandra et al. had demonstrated that the alcoholic extract of flowers has good analgesic activity against hot plate and tail flick method showing central analgesic activity in dose-dependent manner. [68] Another study by Shekawat et al. have also said that a dose of 4-64 mg/kg showed a marked increase in analgesic efficacy on all nociceptive methods. Studies have proposed that the *Madhuca longifolia* is mediated by central or peripheral mechanism.

However, toxicity is also an important parameter which should be evaluated before any drug is used for clinical trials. Mulky et al. have mentioned in their toxicological studies that saponins on parenteral administration are extremely toxic with respect to oral route. The saponin extract from *Madhuca longifolia* was administered in mice orally and the median lethal dose was found to be 1000 mg/kg. Similarly when ethanolic extracts of *Madhuca longifolia* leaves were administered to a male wistar rat for 14 days to observe morphological and histopathological changes, there were no toxic effects on kidney and liver upto a dose of 2000 mg/kg. However, European Food Safety Association reported that excess dose of mahua oil may lead to antifertility effects and European Food Safety Authority.

Madhuca longifolia contain sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides. It is used as an anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer medicine.

MTT assay is the most common method used to determine the cytotoxicity of several drugs. The principle behind MTT assay is that viable cells convert tetrazolium salt MTT into formazan crystals, which can be solubilised for homogenous measurement. Mitochondrial activity for a viable cell remains constant. Therefore, any variation in the number of viable cells is linearly related to mitochondrial activity which in turn is directly proportional to the amount of formazan which is formed. The resulting coloured solution is quantified by measuring the absorbance rate at 500-600 nm.

Conclusion

Madhuca longifolia seed extract has been proven to be more biocompatible than sodium hypochlorite. It is also proven to have good antibacterial properties. *Madhuca longifolia* has the potential to be used as an intracanal irrigant.

Clinical significance

Ideally a root canal irrigant should be biocompatible, nontoxic, and also have a desired smell and palatable taste. Synthetic irrigants, although they are more effective, they are associated with several disadvantages. Such drawbacks have led to the search of natural products with similar efficacy in terms of antimicrobial property, necrotic tissue dissolution and with minimal or no side effects.

Herbal extract from *Madhuca longifolia* with their antimicrobial, anti inflammatory, and therapeutic effects, have the potential to be used as an endodontic irrigant.

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