ORIGINAL RESEARCH PAPER

Volume-8 | Issue-5 | May-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EVALUATION OF WHEAT GRASS JUICE AS A NOVEL STORAGE MEDIUM IN ASSESSING THE PERIODONTAL LIGAMENT CELL VIABILITY TO HBSS AND ALOE-VERA GEL-AN IN VITRO STUDY

Dental Science

Dr C. Rakesh Reddy	Senior Lecturer, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar.
Dr Sravani Nirmala*	Post Graduate Trainee, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar. *Corresponding Author
Dr L. R. Surender	Professor and Head, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar.
Dr M. Narender Reddy	Reader, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar.
Dr Srikanth. P	Associate, Government Medical College Mahabubnagar.
Dr Ankita Reddy Amaravai	Post Graduate Trainee, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar.
Dr Naresh Kumar. K	Senior lecturer, Department of Conservative Dentistry and Endodontics, SVS institute of Dental Sciences Mahabubnagar.

ABSTRACT

BACKGROUND: A spectrum of properties make wheat grass juice as the king of living juices. The aim of the present study is to evaluate and compare Wheat grass juice as a novel storage media in maintaining the periodontal ligament cell viability of dry stored teeth to Aloe-vera gel and Hanks balanced salt solution.

MATERIALS AND METHODS:

Forty therapeutically extracted teeth were randomly divided into four groups according to the storage media. Group 1 (Control group), Group 2 (HBSS), Group 3 (Aloe-vera gel), Group 4 (Wheat grass juice). Teeth were stored for 30 and 60 minutes. The apical 2/3rds of the root surfaces were scraped to obtain residual periodontal ligament cells. The cells were dropped into a falcon tube containing 2.5ml of phosphate buffer solution with super added 0.5mg of type-1 collagenase and incubated for 30mints at 37C,followed by centrifugation for 5minutes at 800rpm.The obtained centrifuged residue was labelled with 0.4% Trypan blue stain. Microscopic evaluation of the cells was done at 100x magnification. The viable cell count was estimated with a haemocytometer and the obtained numerical was statistically analysed using one way analysis of variance complemented by the Tukey test.

RESULTS: There was no significant difference between HBSS and wheat grass juice in maintaining the periodontal ligament cell viability after the first 30minutes of storage.

CONCLUSION: Within the limitations of this study, Wheat grass juice can also be considered as an able storage medium in maintaining the periodontal ligament cell viability alongside HBSS.

KEYWORDS

Wheat grass, periodontal ligament cells

INTRODUCTION:

Traumatic injury to the teeth is a common occurrence in children and adolescents. Avulsion of permanent teeth is the most common dental trauma (1-16% of all the traumatic injuries) occurring in the age group of 8-12yrs (1,4). Management of such teeth is by immediate replantation, which is seldom achieved (2). Therefore, the primary goal is to preserve the viability of periodontal ligament (pdl) cells before the tooth is replanted into the socket (3). The extra oral time and the efficacy of the storage media remains crucial in determining the prognosis of an avulsed tooth (4). A storage medium is a biological formulation that merely resembles an oral environment and preserves the viability of periodontal ligament cells. A probe into the literature review unfolds the existence of various storage media such as, Milk, Viaspan, HBSS, Coconut water, Aloe-vera juice, Gatorade, Saline, Saliva etc. with underlying pros and cons (1, 4-6).

One of the most favoured new super drinks of the 21st century is Wheat grass juice. Among all the green juices, on earth none is more nutritionally powerful than wheatgrass juice which is used to treat many diseases and has powerful alkalizing properties.

It is the young grass of the wheat plant which grows in temperate regions and can survive indoors or outdoors. Growing wheatgrass by soaking the seeds in water and then harvesting the leaves is a common practice

Keeping in view of the dearth and in quest of a newer storage media which is effective in maintaining the pdl cell viability, the present study was aimed at evaluating the efficacy of indigenously prepared wheat grass juice (Triticum aestivum) as a novel storage medium for maintaining the pdl cells viability until the tooth is re-implanted into the socket.

MATERIALS AND METHODS

Forty therapeutically extracted teeth were included in the study. Post extraction measures were taken in a way that the beaks of the forceps doesn't slip beyond the cement-enamel junction in an act for preserving the pdl cells. The pdl from the coronal 3rd of the root surface were scraped off using No 15 Bard Parker (BP) blade to remove possible damaged cells during extraction. The teeth were then bench dried for 15minutes in order to simulate the natural phenomenon. The 40 teeth were then randomly divided (n=10) into three experimental groups based on storage media and a negative control group.

Group 1: HBSS - Commercially available as "Save a tooth".
Group 2: Aloe vera gel

Aloe vera leaves were washed using distilled water. Spiked edges and skin of the leaves were cut using a BP blade no 15. The inner gel was extracted and transferred into a blender. The contents were blended thoroughly and filtered through a strainer (7).

Group 3: Wheat grass juice
One cup of wheat grains were washed and soaked in water overnight. The water was drained and the seeds were sown across the top of a potting soil. Water was sprinkled daily for 7 days. Once the wheat

grass was spilt and the shoots are matured, the wheat grass was cut above the roots with the help of scissors. Washed wheat grass is transferred into a blender. The obtained mix is then strained using a cheese cloth to discard the pulp. The resultant juice is collected and used (16).

Group 4: Control group
Teeth were bench dried and no storage media was used.

METHODOLOGY

All the samples were stored for a stipulated time interval of 30minutes and 60minutes. Later the teeth were handled at the coronal portion of the crown to prevent damage to the pdl cells and were irrigated with phosphate buffered solution to remove any residual storage media. The apical 2/3rd root surface scrapings were dropped into a falcon tube containing 2.5ml of phosphate buffer solution with super added 0.5mg of type-1 collagenase to obtain pdl cells. The tubes were incubated for 30minutes at 37C and then subjected to centrifugation for 5minutes at 800rpm. The supernatant was discarded using a micropipette, and the obtained centrifuged residue was labelled with 0.4% trypan blue stain. Following staining, microscopic evaluation of the cells were done at 100x magnification. The number of cells viable and non-viable were determined by counting the cells overlying a 4x1mm^2 area of the Neubauer chamber.

The viable cell percentage was calculated as

$$\frac{\text{Total cells} - \text{stained cells} \quad \times 100}{\text{Total cells}}$$

STASTISTICALANALYSIS:

The obtained data was quantified and statistically analysed using one way analysis of variance complemented by the Tukey test.

RESULTS:

Results showed that the mean viable cells in the first 30minutes were high for HBSS followed by wheatgrass juice, aloe vera gel and least was with control group. Inter-group analysis revealed statistically significant value (p-value=0.00001) however statistical significance achieved among HBSS and wheat grass juice falls negligible. (Table 1) The mean viable cells in the next 60minutes were high in HBSS followed by aloe vera gel, wheatgrass juice and control group. Tukey pair wise comparison achieved statistical significance among groups (p-value of 0.00001). There was statistically significance difference among HBSS and wheat grass (Table 2).

TABLE-1

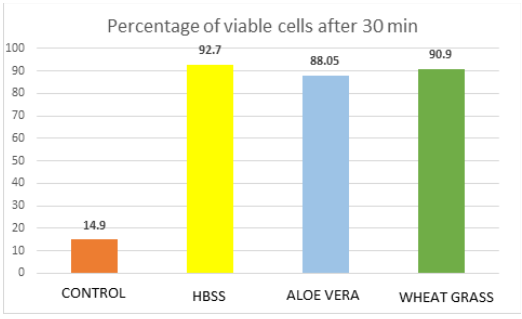
Group		Viable 30	Viable 60
control	Mean	2.0000	0.4000
	Std. Deviation	0.70711	0.54772
HBSS	Mean	13.0000	11.6000
	Std. Deviation	1.87083	0.54772
Aloe vera	Mean	11.8000	11.0000
	Std. Deviation	0.83666	0.70711
wheat grass	Mean	12.0000	9.6000
	Std. Deviation	0.70711	0.54772
F value		102.410	391.381
P value		**<0.0001****	**<0.0001****

**p value<0.01(highly significant)

TABLE-2

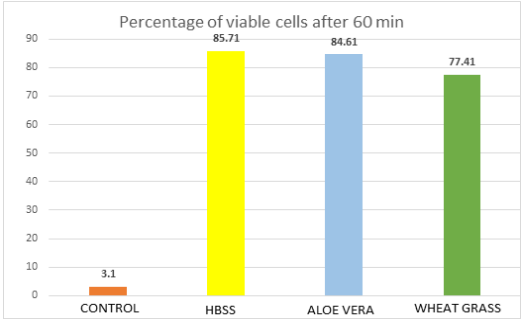
Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.
viable30	control	HBSS	-11.00000*	.72111	<0.0001**
		Aloe vera	-9.80000*	.72111	<0.0001**
		wheat grass	-10.00000*	.72111	<0.0001**
	HBSS	Control	11.00000*	.72111	<0.0001**
		Aloe vera	1.20000	.72111	0.373
		wheat grass	1.00000	.72111	0.525
	Aloe vera	Control	9.80000*	.72111	<0.0001**
		HBSS	-1.20000	.72111	0.373
		wheat grass	-.20000	.72111	0.992
	wheat grass	Control	10.00000*	.72111	<0.0001**
		HBSS	-1.00000	.72111	0.525
		Aloe vera	.20000	.72111	0.992
viable60	control	HBSS	-11.20000*	.37417	<0.0001**
		Aloe vera	-10.60000*	.37417	<0.0001**
		wheat grass	-9.20000*	.37417	<0.0001**
	HBSS	control	11.20000*	.37417	<0.0001**
		Aloe vera	.60000	.37417	0.404
		wheat grass	2.00000*	.37417	.000
	Aloe vera	control	10.60000*	.37417	.000
		HBSS	-.60000	.37417	.404
		wheat grass	1.40000*	.37417	.009
	wheat grass	control	9.20000*	.37417	.000
		HBSS	-2.00000*	.37417	.000
		Aloe vera	-1.40000*	.37417	.009

**p value<0.01(highly significant)



BAR DIAGRAM-1

Bar diagram 1 depicting pdl cell viability in various storage media after 30minutes.Though the *observation revealed numerical statistical differnce among HBSS and Wheat grass juice it falls negligable*.*



BAR DIAGRAM-2

Bar diagram 2 depicts a group comparison which attained a statistical significance. *Observations include a decline in the viable cell count in Wheat grass to HBSS*.*

DISCUSSION:

Avulsion injuries are the worst of the dento-alveolar injuries (2). Post trauma the presence of life endangering injuries needing immediate medical attention or non-availability of a dental care nearby delays immediate replantation of the avulsed tooth. Hence there is a situation that entitles the need of a storage medium which preserves the health of pdl cells until the tooth is replanted back into the alveolar socket (2, 3, 5). The success of such tooth is directly related to the remaining count of viable pdl cells (3). A viable storage medium is a prime requisite to maintain cell viability.

The fate of a replanted tooth is governed by the extra-oral time and the medium in which it is stored (2). Teeth that were replanted within 30 minutes had a convincing prognosis when compared to greater extra-oral time, marking the significance of extra oral time (1, 3, 4). Experimental studies laid emphasis even on the storage media and postulated it as a crucial prognostic factor (3,10, 11).

On an average it takes about 10 to 15minutes for an injured person to recover from the traumatic event and act appropriately. In the present study, to simulate such situation the extracted tooth were bench dried for 15minutes (7).

Trypan blue exclusion assay quickly and easily differentiates non-viable from viable cells. The damaged cells take up the blue stain and the viable cells remain colourless or pink .To quantitate the number of viable cells in the current study and to preserve maximum cell viability, the cells were treated with type-1 collagenase for rapid cell retrieval and cellular integrity (7,9).

HBSS is a non-toxic solution with balanced pH. Though it is recommended by the International Association of Dental Traumatology its usage is hindered on economic grounds (6-8, 13)

Aloe vera gel contains more than 75 ingredients which includes water (98%) and active compounds which have antibacterial, anti-inflammatory, antioxidant, immune bosting and hypoglycaemic properties (7) hence it was suggested as a suitable storage medium. (8, 11)

In the present study the number of viable cells in the first 30minutes and next 60minutes were high for both HBSS and aloe vera gel when compared to control group which is in accordance to a study done earlier. (1,3,7,8)

Number of viable cells during 30minutes and 60minutes were higher for Wheat grass juice when compared to control group. Wheat grass juice is a natural source of vitamins and minerals. It has a complete source of protein, supplying all of the essential amino acids. It is chemically active and contains over 80 enzymes such as protease, amylase, lipase etc.

For the first 30 minutes and 60 minutes, HBSS demonstrated higher viable cells when compared to Aloe vera gel. The results obtained were statistically significant ($p<0.05$) and were in accordance to the studies done earlier (8, 15).

After 30 minutes, the number of viable cells were higher in HBSS to wheat grass and wheat grass to aloe vera gel. The results were statistically significant ($P<0.05$)

After 60 min of storage, there was gradual decline in number of viable cells in all the groups when compared to 30 minutes of storage. But this decline was higher in Wheatgrass juice when compared to the other experimental groups. The results were statistically significant ($p < 0.05$). This may be due to the oxidation of nutrients in wheatgrass juice when exposed to air.

Wheatgrass juice is the king of living juices because of its antibacterial, anti-inflammatory, anti-hypertensive, anti-carcinogenic, detoxifier, immune boosting properties. It is particularly high in 70% chlorophyll and considered as green blood, which cleanses and builds the blood. It is the first product of light and therefore contains more healing properties than any other element. It builds RBC which carry oxygen to every cell and maintains cellular function at an optimal level in highly oxygenated environment. It has the ability to repair and heal the damaged cells. The extract has high content of Bioflavonoids which include Apigenin, Quercitin, Luteonin, (16-18).

Results showed that wheat grass juice was equally effective as HBSS in maintaining the pdl cells viable in the first 30minutes and there was gradual decrease in viable cells in the next 60minutes when compared to HBSS and Aloe vera gel.

CONCLUSION:

Within the limitations of this study it can be concluded that wheatgrass juice could be a suitable, alternate storage medium for avulsed tooth. Ease of availability, less preparation time and high economicity makes it more congenial for use. The spectrum of properties like high nutritious content, enzymes, vitamins, minerals, proteins not only maintains cellular function at an optimal level but also a highly oxygenated environment with the richness of chlorophyll has an antibacterial property which has the ability to repair and heal the damaged cells.

Furthermore, in the research arena more studies have to be instituted in establishing wheat grass juice as an effective storage media.

REFERENCES

1. Mahal NK, Singh N, Thomas AM, Kakkar N. Effect of three different storage media on survival of periodontal ligament cells using collagenase-dispase assay. Int Endod J. 2013 Apr; 46(4):365-70.
2. Gopikrishna V, Thomas T, Kandaswamy D. A quantitative analysis of coconut water: a new storage media for avulsed teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Feb; 105(2):e61-5.
3. Ozan F, Polat ZA, Er K, Ozan U, Değer O. Effect of propolis on survival of periodontal ligament cells: new storage media for avulsed teeth. J Endod. 2007 May; 33(5):570-3.
4. Kokkali VV1, Bendgude V2, Sharangpani G. Comparative evaluation of post-traumatic periodontal ligament cell viability using three storage media. Eur Arch Paediatr Dent. 2017 Jun; 18(3):209-214.
5. Harkacz OM Sr, Carnes DL Jr, Walker WA 3rd. Determination of periodontal ligament cell viability in the oral rehydration fluid Gatorade and milks of varying fat content. J Endod. 1997 Nov; 23(11):687-90.
6. Hwang JY1, Choi SC, Park JH, Kang SW. The use of green tea extract as a storage medium for the avulsed tooth. J Endod. 2011 Jul; 37(7):962-7.
7. Sharma M1, Sharma S2, Reddy YG3, Mittal R4, Agarwal V5, Singh C4, Singh A6. Evaluation of Periodontal Ligament Cell Viability in Three Different Storage Media: An in Vitro Study. J Dent (Tehran). 2015 Jul; 12(7):524-31.
8. Babaji P1, Melkundi M2, Devanna R3, Suresh BS4, Chaurasia VR5, Gopinath PV6. In vitro comparative evaluation of different storage media (hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. Eur J Dent. 2017 Jan-Mar; 11(1):71-75.
9. Sanghavi T1, Shah N, Parekh V, Singbal K. Evaluation and comparison of efficacy of three different storage media, coconut water, propolis, and oral rehydration solution, in maintaining the viability of periodontal ligament cells. J Conserv Dent. 2013 Jan; 16(1):71-4.
10. Pearson RM1, Liewehr FR, West LA, Patton WR, McPherson JC 3rd, Runner RR. Human periodontal ligament cell viability in milk and milk substitutes. J Endod. 2003 Mar; 29(3):184-6.
11. Badakhsh S1, Eskandarian T2, Esmaeilpour T3. The use of aloe vera extract as a novel storage media for the avulsed tooth. . Iran J Med Sci. 2014 Jul; 39(4):327-32.
12. de Souza BD1, Bortoluzzi EA, da Silveira Teixeira C, Felippe WT, Simões CM, Felippe MC. Effect of HBSS storage time on human periodontal ligament fibroblast viability. Dent Traumatol. 2010 Dec; 26(6):481-3.
13. Fulzele P1, Baliga S1, Thosar N1, Pradhan. Evaluation of Aloevera Gel as a Storage Medium in Maintaining the Viability of Periodontal Ligament Cells - An in Vitro Study. J Clin Pediatr Dent. 2016 Winter; 40(1):49-52.
14. Goswami M1, Chaitra T, Chaudhary S, Manuja N, Sinha A. Strategies for periodontal ligament cell viability: An overview.J Conserv Dent. 2011 Jul; 14(3):215-20.
15. Fulzele P, Baliga S, Thosar N, Pradhan D. Evaluation of Aloevera gel as a storage medium in maintaining the viability of periodontal ligament cells – An in vitro study. J Clin Pediatr Dent 2016;40:49-52.
16. Bar-Sela G, Cohen M, Ben-Arye E, Epelbaum R. The Medical Use of Wheatgrass: Review of the Gap Between Basic and Clinical Applications. Mini Rev Med Chem. 2015;15(12):1002-10
17. Parit SB, Dawkar VV, Tanpure RS, Pai SR, Chougale AD. Nutritional Quality and Antioxidant Activity of Wheatgrass (Triticum aestivum) Unwrap by Proteome Profiling and DPPH and FRAP assays. J Food Sci. 2018 Aug;83(8):2127-2139
18. Bhat NA, Wani IA, Hamdani AM, Gani A. Effect of extrusion on the physicochemical and antioxidant properties of value added snacks from whole wheat (Triticum aestivum L.) flour. Food Chem. 2019 Mar 15;276:22-32.