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Evaluation of Efficacy of Glycyrrhiza Glabra in the Management of Oral Submucous Fibrosis - A Preliminary Study

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Abstract

Background: Oral submucous fibrosis is a chronic, insidious, progressive disease of the oral cavity strongly associated with areca nut chewing habit. Till date no conclusive management protocol is stand still in Oral submucous fibrosis, but there is always a constant research to explore pathogenesis and management. Hence attempt was made to evalaute the efficacy of glycyrrhiza glabra in the management.

Objectives: Efficacy of glycyrrhiza glabra was scientifically evaluated by estimating serum antioxidant activity status and malondialdehyde levels before and after treatment. To evaluate the improvement in clinical parameters after treatment with glycyrrhiza glabra.

Methods: Twenty oral submucous fibrosis patients were included in the study. All the patients had taken two 500mg glycyrrhiza glabra capsules thrice a day for three months. Serum antioxidant activity status and malondialdehyde was estimated in patients before and after therapy. Statistical analysis was done using ANOVA, paired‘t’ test and unpaired‘t’ test.

Results: Improvement in burning sensation, mouth opening and tongue protrusion were statistically highly significant ($p<0.001$). Serum antioxidant activity status was significantly improved after therapy and malondialdehyde level was reduced after therapy.

Conclusion: Glycyrrhiza glabra proved to be relatively safe, noninvasive, economical and efficatious treatment with improvement in signs and symptoms thereby enhances the patients compliances. Improvement in serum antioxidant activity status and malondialdehyde level intimates the role of oxidative stress in the pathogenesis of oral submucous fibrosis as well determines the antioxidant properties of Glycyrrhiza glabra.

Keywords: *Oral submucous fibrosis, glycyrrhiza glabra, antioxidants, Antioxidant activity, Malondialdehyde.*

Introduction

Oral submucous fibrosis (OSMF) is an enigmatic disease though it is easy to diagnose, management of the disease is still palliative not curative. OSMF is a chronic, insidious, potentially malignant disorder which is strongly associated with betel nut chewing.[1, 2] The current status of focus is on oxidative stress with altered oxidants and antioxidants levels as one of the contributing factor in the pathogenesis. [3, 4]

Management of the disease is empherical and unsafisfactory and it is still a challenge for oral health care workers to treat this premalignant condition. Ayurvedic medicine is a system of traditional medicine native to

India and is a form of complementary and alternative medicines (CAM). [5] There are ennumerable evidence in the literature that, many incurable mucosal diseases are very well managed by using ayurvedic medicines. Glycyrrhiza glabra is one of the most widely used herbs from the ancient medical history of Ayurveda. Some of the components like glycyrrhizin, flavonoids and isoflavonoids etc are proved to possess antioxidant, anti inflammatory, immunomodulatory and anti carcinogenic properties.[6] Has been successfully used in the treatment of oral lichen planus, which is also considered as one of the oral premalignant condition.[7]

All such properties of glycyrrhiza glabra and evidence of its successful use in medicine has encouraged us the possible benefits of its use in OSMF. Hence present study was planned to evaluate the efficacy of glycyrrhiza glabra in the management of OSMF and to scientifically assess the potential benefits of glycyrrhiza glabra by measuring serum antioxidant activity (AOA) status and Malondialdehyde (MDA) levels before and after treatment. To the best of our knowledge, this is the first study where total AOA status in serum is estimated in OSMF patients and also first study where glycyrrhiza glabra is used in the management of OSMF.

Materials and Methods

Present preliminary study was carried out in the Department of Oral Medicine and Radiology, College of Dental Sciences, Davangere, Karnataka, India. Twenty OSMF subjects of either sex were enrolled in the study with following criteria.

Inclusion Criteria

1. Patients with OSMF diagnosed clinically and histopathologically.
2. Patients who had not taken any treatment earlier for OSMF.
3. Patients who are ready to quit the habit of gutkha chewing and other habits and are willing for regular follow ups.
4. Patients under the age of 40 years.

Exclusion Criteria

1. OSMF patients with coexisting systemic illness and patients under treatment with systemic steroids, anti hypertensive agents, digoxin, diuretics and estrogen based oral contraceptives.
2. History of hypersensitivity to glycyrrhiza glabra.
3. Pregnant women and lactating mothers.

A detailed case history was taken and a thorough clinical examination was done and recorded on a standard pro forma. A formal ethical clearance to conduct the study was obtained from the institution committee and written informed consent was obtained from all the subjects. Following clinical parameters were noted down for each patient

Burning sensation was measured using visual analogue scale (VAS) by asking a patient to mark on a point which best represented his/her burning sensation on a pro forma with marking from 0 to 10 with interval of 1cm, zero being the no burning and 10 being worst possible burning sensation and burning sensation was designated by percentage (%). Mouth opening was measured using vernier caliper from mesioincisal angle of maxillary central incisor to mesioincisal angle of mandibular central incisors and was designated by millimeter (mm). Clinical staging was done based on mouth opening into Stage I: > 40 mm, Stage II: 31-40mm, Stage III: 21-30mm, Stage IV: 11-20mm, Stage V: 0-10mm. Tongue protrusion was measured using divider and scale from tip of the tongue on maximum protrusion to mesioincisal angle of the maxillary central incisor and was designated by millimeter (mm). Cheek flexibility was measured according to the

method proposed by Mathur and Jha as quoted by Bailoor and Nagesh and designate by centimeter (cm).[8]

All the patients were subjected for routine hematological examination and biopsy, followed by histopathological grading was done according to Utsunomiya H et al, 2005.[9]

Glycyrrhiza glabra raw drug powder was obtained from Alva pharmacy, Yograj Arogyadhama, Dakshina kannada, Karnataka, India and Zero sized empty capsules were obtained from Rajesh chemicals Co, Direct importers and Stockists, Mumbai, India. 500mg Glycyrrhiza glabra capsules were fabricated using semi automated capsule filling machine under aseptic conditions in the Department of Pharmaceuticals, College of pharmacy, Davangere. Ninety capsules essential for the duration of 15 days were packed in air tight plastics boxes and stored under refrigerator until delivered to patients.

Before initiation of therapy, from each individual 5 ml of blood was collected using aseptic measures, samples were centrifugated at 3000 RPM for 10 minutes and serum was split in to separate portions immediately after blood centrifugation. Serum was used for MDA and AOA analysis immediately or stored in small aliquots with caps at -20^0C for maximum of one month until assayed. MDA was estimated using thiobarbituric acid (TBA) method and AOA was estimated using principle of thiobarbituric acid reactive substances by means of calorimetric method. [10,11]

Patients were counseled to quit the habit and advised to take two 500mg Glycyrrhiza glabra capsules thrice a day orally after food for 3 months and they were recalled every 15 days and clinical parameters were noted down. After three months of treatment again AOA & MDA were estimated using above mentioned methods.

Statistical analysis: Data analysis was done using statistical package for social science (SPSS, V 10.5). Student t test and ANOVA test were used for statistical analysis. Results were expressed as Mean ± SD and "p"value of less than 0.05 was considered as statistically significant.

Results

Age range of the study population was 18-38years with the mean age of 25.7 years. Main stream of our patients were in third decade of life. All the patients participated in the study were males. It was noticed that clinically 4(20%) patients were in stage I and 6(30%), 8(40%) and 2(10%) were in II, III and stage IV respectively. According to histopathological staging, 5(25%) were in early stage , 7(35%) and 8(40%) belonged to intermediate stage and advanced stage respectively.

Burning sensation (Table 1): The mean VAS at baseline was 50.3±23.0% and was in the range of 90% to 10 %. There was gradual reduction in burning sensation from base line through 6th visit. Percentage of reduction in the burning sensation from base line to 1st, 2nd, 3rd , 4th , 5th and 6th visits were 15%, 49%,71%, 89%,96% and 92%, respectively. Reduction in burning sensation was statistically significant in the 1st visit (p=0.012) and statistically highly significant from 2nd to 6th visit (p< 0.001) with maximum reduction in burning sensation at 5th visit (96%). There was 4% increase in burning sensation from 5th to 6th visit (96% to 92%). There was statistically significant improvement in burning sensation in clinical stage I & II and improvement was highly significant in stage III (p<0.001), there was clinical improvement in stage IV but, observations were statistically non significant (Table2).

Mouth opening (Table 1): Range of pretreatment mouth opening in 20 patients was from 20mm to 47mm with a mean of 31.6± 8.9 mm. Improvement in mouth openings as compared to base line in 1st, 2nd, 3rd ,4th,5th and 6th visits were 0.15 mm, 1.10mm, 0.45mm, 0.80mm, 1.05mm and 1.35mm respectively. Improvement in the mouth opening was statistically significant (p=0.002) in 4th visit and

statistically highly significant from 5th visit onwards with p<0.001. Maximum improvement in mouth opening from base line to last visit was 5 mm with mean maximum increase of 1.35mm at 6th visit. Clinical improvement of mouth opening noted in stage II and III was statistically significant with a p value of 0.017 and 0.002 respectivelly. However increase in mouth opening in stage I and Stage IV were statisticcaly non significant (Table 2).

Tongue protrusion (Table 1):The mean tongue protrusion of 20 patients at baseline was 43.8±13.9mm with a range of 21 mm to 70mm. Increase in tongue protrusion was statistically significant at 5th visit (p=0.05) and statistically highly significant at 6th visits with p<0.01. Maximum increase in tongue protrusion was 2mm with mean maximum increase of 0.5mm. Increase in tongue protrusion was statistically significant in stage III, however improvement in other clinical stages was non significant (Table 2).

Cheek flexibility (Table 1): Cheek flexibility of the patients at base line was ranging from 0.1cm to 0.3 cm with mean of 0.19cm. There was no improvement in cheek flexibility from baseline to 4th visit, there was increase of 0.01cm in 5thvisit and 0.02cm in 6th visit, however improvement was statistically non significant even in different clinical stages (Table 2).

The mean Serum AOA status in OSMF patients before the treatmnet was 1.51±0.52 mmol/L (mean±SD) which was increased to 1.80±0.39 mmol/L (mean±SD) after treatment. Improvement in AOA staus was statistically highly significant (p<0.001). Serum AOA status was also compared between different clinical stages before and after therepy, however difference was statistically non significant (Table 3).

The level of MDA before treament was 3.60±0.51 nmol/ml (mean±SD) and there was reduction in MDA levels to 2.43±0.56 nmol/ml (mean±SD) after treatment, diffrence was statistically highly significant (p<0.001). However statistically no diffrence was noted before and after therapy between different clinical stages (Table 3).

There were no reported instances of side effects or intolerance to glycyrrhiza glabra. Only two patients developed symptoms of gastritis for 2-3 days after in take of medication, However, they were able to tolerate the drug in the following visits and had no further complaints and reported amelioration of symptoms.

Discussion

Management of disease is obligatory considering the mortality and morbidity caused by the disease. So far documentation revealed that none of the management strategies are stand still in OSMF, but there is always a constant research to explore pathogenesis and management. In the present study glycyrrhiza glabra was biochemically eveluated for its efficacy in managing OSMF by estimating serum AOA staus and MDA levels.

All the patients in the study were males (100%), accordance with other studies with male predominance.[12, 13,14] Age range of the study population was 18-38years with the mean age of 25.7 years. Main stream(80%) of our patients were in third decade of life. Finding were coinciding with study by Maher et al, who reported 70% patients in third decad.[15] Another study by Borle and Borle reported majority of patients under the age of 30 years.[16]

Burning sensation was the most consistent symptom in OSMF patients noticed in the present study. Mean pecentage of improvement noted in burning sensation was 92% at the end of the therapy. In present study 17(85%) of our patients had complete resolution of burning sensation, where as on an average there was 2% increase in burning sensation from 5th to 6th visit that is because 30% of burning sensation was remaining in one patient at 6th visit and two patients had increase in burning sensation

from 10% in 5th visit to 20% & 30% in 6th visit respectively. Increase in burning sensation may be attributed to reuse of areca nut by 2 of our patients. Lai Dr et al followed up 150 osmf patients for 10 years and noted that group A- 25 patients received vitamin B complex tablets, buflomedial hydrochloride, topical triamcinolone acetonide and group B- 25 patients received biweekly submucosal injection of a combination of dexamethasone and hyaluronidase and group C -25 patients recieved combination of medications from group A and B. Reduction in burning sensation in group A was 88%, group B was 89% and group C was 91%.[17] Another study in the year 1988 Gupta D and Sharma S C noted that a biweekly submucosal injection of 2ml placental extracts for a period of 10 weeks showed 51% improvement in the burnirng sensation.[18] Consistent improvement in burning sensation in our study clearly indicates that systemic administration of the glycyrrhiza glabra with strong antioxidant, anti inflammatory, immunomodulatory properties in a single drug is essential in managing the disease.

The clinical improvement in burning sensation may be attributed to stoppage of habit and strong anti-inflammatory action of glycyrrhiza glabra. Its constituents exhibit steroid like properties by inhibiton of phospholipase A2 activity and inhibition of enzyme 11-β hydroxy steroid dehydrogenase significantly increases the levels of cortisol and also stimulation of the glucocorticoid receptors. Addede to it glycyrrhiza glabra possess strong antioxidant activity, antiallergic ativity and found to inhibit mitochondrial lipid peroxidation.[19]

The mean improvement in mouth opening is 1.35mm. One of our patient showed maximum of 5mm improvement where as four patients did not show any improvement but there was no further worsening of the condition. Finding were in concurent with study by Gupta ds et al. [20] Improvement in mouth opening was clinically & statistically significant but non satisfactory. The probable reason for improvement in mouth opening may be due to decrease in the oral soreness secondary to antiinflamatory effects of glycyrrhiza glabra.

The mean increase of tongue protrusion of 0.04mm was noted in our study was in accordance with other studies.[4.21] eventhough marked improvement in tongue protrusion was not seen in our patients, it is definitely noteworthy that there was some improvement after treatment. Slight improvement in cheek flexibility noted in the later visits is remarkable but clinically not significant. Observations of our study can not be correlated with other studies since to best of our knowledge there is no published data exits, where cheek flexibility is measured and compared after any kind of therapy

Marked improvement was noted in terms of serum AOA after treatment, indicates the role of oxidative stress in the disease, antioxidant properties of glycyrriza glabra and antioxidant supplements required for the managemnet of the disease. Direct correlation can not be drawn since our study is prelinimary and stage wise comparison was not done before and after therapy due to lack of published data to correalate, but these observations are in accordance with the other study where increase in serum β carotene and serum vitamin E were noted in OSMF patients after administration of antoxid tablets.[4] Improvement may be attributed to Significant antioxidant properties as demonstrated by Glycyrrhizin and glabridin, which inhibit the generation of ROS by neutrophils at the site of inflammation.[19] Addead to it with the improvement in burning sensation patients were able to consume food with minimal discomfort thus dietary antioxidant component may also play role in increased serum antioxidant after therapy as well indicates possible improvement in the disease process.

Gupta et al in the year 2004 estimated MDA in osmf patients after administartion of antoxid thrice dialy for 6 weeks and found that there was marked reduction in serum MDA levels after treatmnet. [4] Simillar results were prominent in our study too, where marked reducction in serum MDA was noted after treatment. Decrease in MDA levels can be related to capacity of glycyrrhiza glabra to inhibit mitochondrial fraction of lipid perroxidation and scavenging activity against nitric oxide, superoxide, hydroxyl radicals.[19] And also it can be due to improvement in AOA status.

Conclusion

Since the pathogenesis is still obscure, and the disease, once established, is not reversible, alleviation of the symptoms and enhancement of the living conditions of patient shape the basis for its management. Glycyrrhiza glabra being the most widely used herb due its ennoble properties like strong antiinflammatory, antioxidant, immunomodulation, antiallergic as well as its anticarcinogenic activity has demonstrated its potential advantages in treating this chronic unremmitent, immunologically mediated disease. Considering the significant improvements in the symptoms as well as objective signs of the condition, glycyrrhiza glabra was seen to be efficacious, reliable, noninvasive & safe drug in the management of OSMF. Thus glycyrrhiza glabra should be considerd in the managemnet along with the other treatment modalities. Further trials in this regard should be carried out in a large sample size with longer follow ups to investigate the probable mechanisms by which glycyrrhiza glabra exerts this beneficial effect as well as the effects of supplementation with other drugs and dosages. Further studies should be carried out to asess the link between oxidative stress in OSMF and its role in malignant transformation of the disease.

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Legends Table

Table 1: Comparision Of Clinical Parameters During Treament From Base Line To 6th Visit

Time interval	Burning sensation (%)			Mouth opening (mm)			Tongue protrusion (mm)			Cheek flexibility (cm)		
	Mean difference ± SD	Difference from base line (%)	T* P value	Mean difference ± SD	Difference from base line	T P value	Mean difference ± SD	Differen ce from baseline	T P value	Mean difference ± SD	Difference from base line	T P value
Baseline	50.3±23.0	-	-	31.6± 8.9	-	-	43.8±13.9	-	-	0.19±0.07	-	-
1st visit	43.0±24.1	7.3(15)	2.79 0.012,S	31.7±8.7	0.15	1.00 0.33	43.7±13.9	0.03	1.00 0.33	0.19±0.07	0.00	1.00 0.33
2nd visit	26.8±21.5	24.4(49)	8.07 <0.001,HS	32.7±8.6	1.10	1.29 0.21	43.8±13.9	0	0 1.0	0.19±0.07	0.00	1.00 0.33
3rd visit	15.0±17.9	35.3(71)	7.02 <0.001,HS	32.0±8.9	0.45	2.44 0.03	43.8±14.0	0	0 1.0	0.19±0.07	0.00	1.00 0.33
4th visit	5.5±9.3	44.8(89)	10.23 <0.001,HS	32.4±8.7	0.80	3.56 0.002,S	44.0±14.0	0.02	1.71 0.10	0.19±0.07	0.00	1.00 0.33
5th visit	2.0±7.0	48.3(96)	10.28 <0.001,HS	32.6±8.4	1.05	4.70 <0.001, HS	44.1±13.9	0.03	2.0 0.05,S	0.20±0.08	0.01	1.45 0.16
6th visit	4.0±9.9	46.2(92)	10.52 <0.001,HS	32.9±8.3	1.35	4.76 <0.001, HS	44.2±13.8	0.04	2.99 <0.001,HS	0.20±0.08	0.02	1.83 0.08

*= Paired t test, SD= Standard deviation, S= Significant, HS= Highly significant

Table 2: Clinical Parameters Before And After Treatment In Different Clinical Stages

Clinical stage	Burning sensation (%)			Mouth opening (mm)			Tongue protrusion (mm)			Cheek flexibility (cm)		
	Before	After	D* T** P	Before	After	D T P	Before	After	D T P	Before	After	D T P
I	30.0±21.6	7.5±13.0	42.5 8.88 0.003,S	45.5±1.0	46.3±1.3	0.8 1.57 0.22,NS	62.3±6.18	62.5±6.5	0.3 1.00 0.39,NS	0.20±0.08	0.20±0.08	0.0 0.0 1.00,NS
II	45.8±30.4	5.0±12.2	40.8 3.57 0.02,S	34.7±3.5	36.2±3.3	1.5 3.50 0.017,S	51.5±1.6	51.8±1.8	0.3 1.58 0.18,NS	0.22±0.008	0.23±0.05	0.01 1.00 0.36,NS
III	32.5±19.1	2.5±7.1	30.0 10.0 <0.001,HS	25.1±1.5	26.3±1.3	1.2 4.97 0.002,S	33.1±5.4	33.8±5.7	0.07 2.38 0.04,S	0.18±0.07	0.20±0.09	0.02 1.53 0.17,NS
IV	55.0±22.9	0.0	55.0 22.0 0.27,NS	20.0±0.0	24.0±0.0	4.0 4.0 0.16,NS	25.5±6.4	26.0±7.1	0.05 1.00 0.50,NS	0.10±0.0	0.10±0.0	00 00 1.00,NS
V	-	-	-	-	-	-	-	-	-	-	-	-
Overall	30.3±22.9	4.0±9.9	46.3 10.52 <0.001,HS	31.6±8.9	33.0±8.3	1.4 5.25 <0.001,HS	43.7±13.9	44.2±13.8	0.5 3.33 0.004,S	0.19±0.07	0.20±0.08	0.01 1.83 0.08,NS

*= Difference from baseline , **= Paired t test

Table 3: Serum Aoa Status And Mda Levels Before And After Treament In Different Clinical Stages.

Clinical stage	AOA(mmol/L)					MDA(nmol/ml)				
	Before	After	D*	T**	P	Before	After	D	T	P
I	1.45±0.38	1.64±0.36	0.19	0.55	0.62,NS	3.64±1.31	2.46±0.60	1.18	1.41	0.25,NS
II	1.44±0.42	1.77±0.48	0.33	1.63	0.16,NS	2.86±0.31	2.24±0.47	0.62	2.66	0.04,S
III	1.58±0.70	1.97±0.37	0.39	1.39	0.21,NS	4.15±0.72	2.45±0.65	1.70	6.00	0.001,S
IV	1.59±0.47	1.54±0.06	0.04	0.11	0.19,NS	3.54±0.82	2.81±0.46	0.73	2.87	0.21,NS
V	-	-	-	-	-	-	-	-	-	-
Overall	1.51±0.52	1.80±0.39	0.29	5.24	<0.001, HS	3.60±0.51	2.43±0.56	1.17	5.24	<0.001, HS

*= Difference from baseline , **= Paired t test