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DISCOVERING THOUGHTS AND INVENTING FUTURE

Highlights

- Intralesional Dexamethasone
- Spermogram of Infertile Men
- Lemongrass Oil Mouthwash
- Formulation of Paracetamol

The Blood Plasma

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Comparative Study of Intralesional Dexamethasone, Hyaluronidase & Oral Pentoxifylline in Patients with Oral Submucous Fibrosis

By Anjum Aara Satishkumar GP C Vani , Venkat Reddy M , Sreekanth K & Ibrahim M

Department of Oral Medicine and Radiology,Sri Sai College of Dental Surgery

Abstract - *Back ground :* Oral submucous fibrosis (OSF) is characterized by excessive production of collagen leading to inelasticity of the oral mucosa and atrophic changes of the epithelium.

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Keywords : *Oral submucous fibrosis, pentoxifylline, Dexamethasone, Hyaluronidase Lignocaine, burning sensation, mouth opening, tongue protrusion, cheek flexibility.*

GJMR-L Classification : *NLMC Code: WB 350,WU 158, WU 113, WU 101.5*

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Comparative Study of Intralesional Dexamethasone, Hyaluronidase & Oral Pentoxifylline in Patients with Oral Submucous Fibrosis

Anjum Aara ,[α] Satishkumar GP,[σ] C Vani [ρ], VenkatReddy M [ω], Sreekanth K [¥] & Ibrahim M [§]

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Results : In the present study, improvement in all the parameters except tongue protrusion were statistically significant ($p < 0.001$), improvement in tongue protrusion was statistically not significant ($p>0.05$).

Conclusion : In the present study, it was found that all the parameters showed significant improvement in dexamethasone group compared to pentoxifylline group. Nevertheless pentoxifylline group also showed statistically significant improvement in all the parameters. For this reason, it is concluded that Pentoxifylline is a good alternative treatment for oral submucous fibrosis in patients in whom dexamethasone is contraindicated, or those who cannot make frequent visits for intralesional injections. In such oral submucous fibrosis patients it is better to substitute pentoxifylline therapy.

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Keywords : Oral submucous fibrosis, pentoxifylline, Dexamethasone, Hyaluronidase Lignocaine, burning sensation, mouth opening, tongue protrusion, cheek flexibility.

I. Introduction

Oral submucous fibrosis (OSF) is a chronic disease of insidious onset featuring the deposition of fibrous tissue in the submucosal layer of the pharynx, palate, fauces, cheek and lips and esophagus.[1] Pindborg and Sirsat have defined OSF as an "insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with epithelial inflammatory reactions followed by a fibro elastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat".[2]

The term oral submucous fibrosis (OSF) follows from oral (meaning mouth), submucosal (meaning below the mucosa of the mouth) and fibrosis (meaning hardening and scarring).[3]

Submucous fibrosis of the oral cavity was first described by Schwartz in 1952 in five Indian females from Kenya and East Africa. Initially the term "atrophia idiopathica mucosae oris" was proposed, which was later replaced by the term currently being used ie, oral submucous fibrosis. The prevalence in India ranges from 0.2 to 0.5percent with a higher predominance in the southern parts of the subcontinent. Individuals between the ages of 20 & 40 years are most commonly affected but cases have been reported in patients ranging from two years to eighty nine years of age.[4]

OSF has a multifactorial etiology. Several factors such as chilli consumption, nutritional deficiency states, areca nut chewing, genetic susceptibility, autoimmunity & collagen disorders have been suggested to be involved in the pathogenesis of the condition.[5]

OSF is a chronic progressive disorder and its clinical presentation depends on the stage of the disease at detection.[4] Clinical features of OSF include

burning sensation on taking spicy food, excessive salivation, dryness of the mouth, defective gustatory sensation and progressive restriction of mouth opening and the protrusion of the tongue.

It is characterized by excessive production of collagen leading to inelasticity of the oral mucosa and atrophic changes of the epithelium.[6] with the progress of the disease, fibrosis may extend from the lamina propria through entire submucosa to the muscle layer. Thick inelastic rope like fibrous bands appear vertically in the buccal mucosa, along the contours of the faucial pillars and around the entire circle of lips thus leading to difficulty in mouth opening and narrowing of the rima oris.[7] The fibrosis also leads to difficulty in mastication, speech and swallowing, pain in the throat and the ears, and a relative loss of auditory acuity due to stenosis of the opening of the eustachian tube.4 The most outstanding feature and the most reliable sign of oral submucous fibrosis is the presence of palpable bands in the oral mucosa, especially in the buccal mucosa. The disease, however, has other characteristic signs, such as diffuse blanching of the mucosa, occurrence of hyperpigmented areas adjacent to zones with loss of pigment, loss of tongue papillae, and a leathery consistency of the mucosa.[8] A more serious complication of this disease is the risk of the development of oral squamous cell carcinoma (SCC), estimated to be 7.6 percent of cases over a 10 year period.

The reasons for the rapid increase of the disease are reported to be due to an upsurge in the popularity of commercially prepared areca nut preparations (pan masala) in india[9] and to an increased uptake of this habit by young people[10] due to easy access, effective price changes and marketing strategies. OSF has been reported in the Indian population and established in the Indian literature since the time of sushruta.[11]

The various treatment modalities proposed for OSF include nutritional support, immunomodulatory drugs, physiotherapy, local drug delivery, combined therapy and surgical management. [12]

Since long, intralesional injections of Dexamethasone (4mg/ml) and Hyaluronidase 1500 IU and 0.5 ml of lignocaine 2% have been the treatment modality in the management of OSF patients with variable success rates in different clinical studies.[13,14]

Pentoxifylline has been recently shown to have significant improvement in the management of OSF patients.[15]

In the course of disease treatment, convenience of drug administration is one of the factor for successful management of disease. As oral administration of drug is more convenient compared to intralesional drug administration, it would be ideal if a oral substitute to intralesional drug delivery is available in the management of OSF.

This study is intended to compare the efficacy of oral Pentoxifylline 400 mg Tablets with intralesional injections of Dexamethasone (4mg/ml) and Hyaluronidase 1500 IU and 0.5 ml of Lignocaine 2% in OSF patients for 12 weeks period of active treatment.

II. Materials and Methods

Dexamethasone Injection 4 mg/ml is a synthetic analogue of prednisolone, having similar but more potent anti-inflammatory therapeutic action and diversified hormonal and metabolic effects. Modification of the basic corticoid structure as achieved in Dexamethasone Injection 4 mg/ml offers enhanced anti-inflammatory effect compared to older corticosteroids. [16]

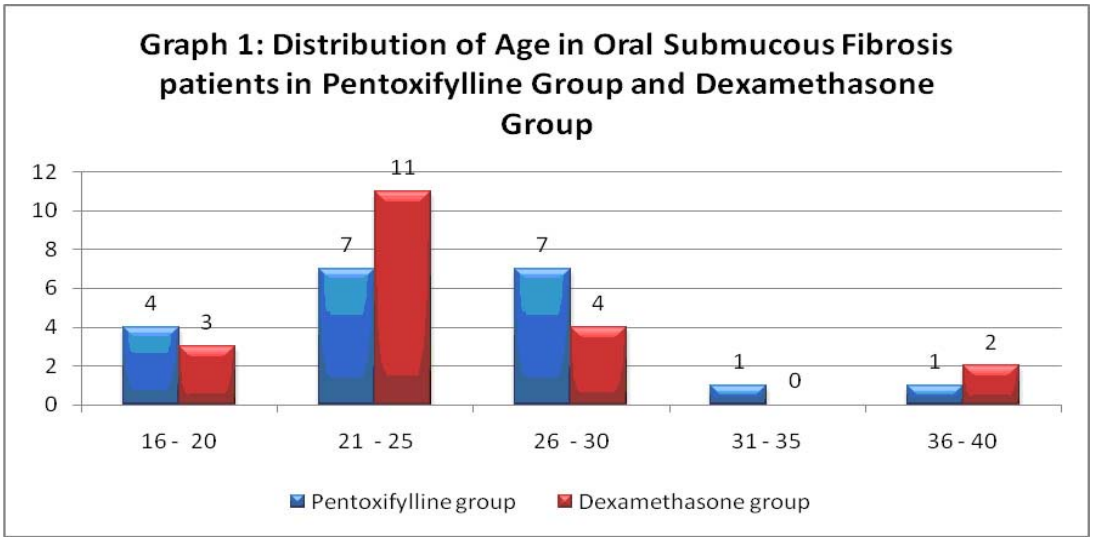
Hyaluronidase is an enzyme which depolymerise the mucopolysacchride hyaluronic acid. It is prepared from the testes and semen of mammals and purified so as to remove most of the inert material. The resulting solution is sterilized and freeze dried. It is a sterile, white or yellowish white powder.

Pentoxifylline is a tri-substituted methylxanthine derivative, the biologic activities of which are numerous. This includes increasing red cell deformability, leukocyte chemotaxis, antithrombin and anti- plasmin activities, and more importantly to the present context, its fibrinolytic activity.

The study population consisted of 40 male patients with OSF. Patients were divided into two groups. 20 patients are in pentoxifylline group and 20 patients in Dexamethasone group. Pentoxifylline Group received oral Pentoxifylline 400 mg tablets twice daily for first 4 weeks and thrice daily for next 8 weeks. Dexamethasone Group received biweekly intralesional injections of Dexamethasone (4mg/ml), Hyaluronidase 1500 IU and 0.5 ml of Lignocaine 2% for a period of 12 weeks. Parameters taken in the study were burning sensation, mouth opening, tongue protrusion and cheek flexibility.

III. Results

40 patients of OSF were selected for the present study and were divided randomly into two groups with 20 patients of OSF in each group. The patients in the first group were given Pentoxifylline orally 400 mg twice a day for 4 weeks initially, followed by 400mg thrice a day for next 8 weeks and the patients in the second group were given intralesional injections of 2ml of Dexamethasone 4mg/ml, 0.5 ml of Lignocaine 1: 80000 and 1500 IU of Hyaluronidase, biweekly for 12 weeks.



The particulars are given in table no. 1 and graph no.1.

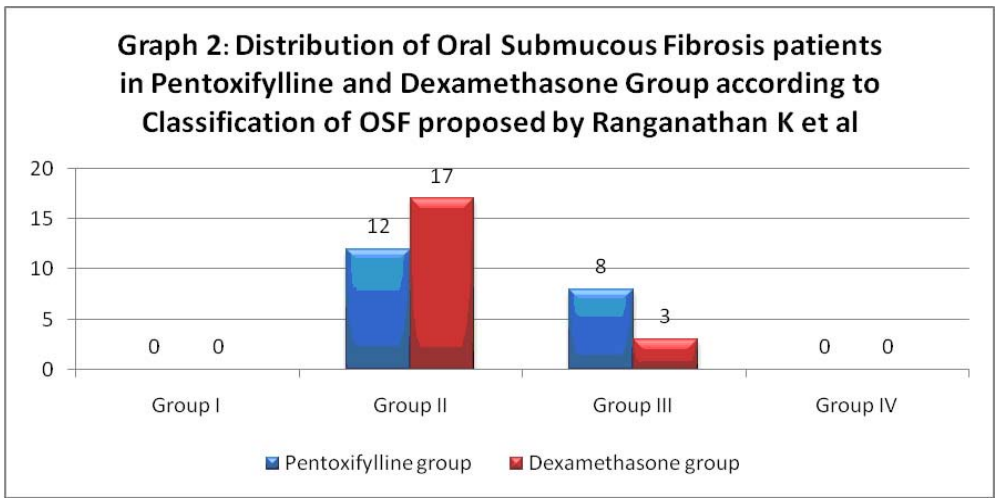
Table 1 : Distribution of Age in Oral Submucous Fibrosis patients in Pentoxifylline Group and Dexamethasone Group.

Age group	Pentoxifylline group	Dexamethasone group
16 - 20	4	3
21 - 25	7	11
26 - 30	7	4
31 -35	1	-
36 -40	1	2
Total	20	20

The age of the 20 patients in the Pentoxifylline group ranged from 18 to 38 years with an average age of 25.05 years. The youngest subject was 18 years old and the oldest was 38 years. Four patients were in the age group of 16-20 years, seven were in the age group of 21 to 25 years and seven were in the age group of 26 to 30 years, one patient in the age group of 31 to 35 years and one patient in the age group of 36 to 40 years. 14 out of 20 patients comprising 70 % were in the age group of 21 to 30 years. The particulars are given in table no. 1 and graph no.1.

The age of the 20 patients in the Dexamethasone group ranged from 18 to 38 years with an average age of 25.15 years. The youngest subject was 18 years old and the oldest was 38 years old. Three patients were in the age group of 16-20 years, eleven were in the age group of 21 to 25 years, four were in the age group of 26 to 30 years, and two patients in the age group of 36 to 40 years. 15 out of 20 patients comprising 75 % were in the age group of 21 to 30 years. The particulars are given in table no. 1 and graph no.1.

All the patients in both, the Pentoxifylline group and the Dexamethasone group were males. These patients were from all the socioeconomic strata.



The particulars are given in table no.2 and graph no.2.

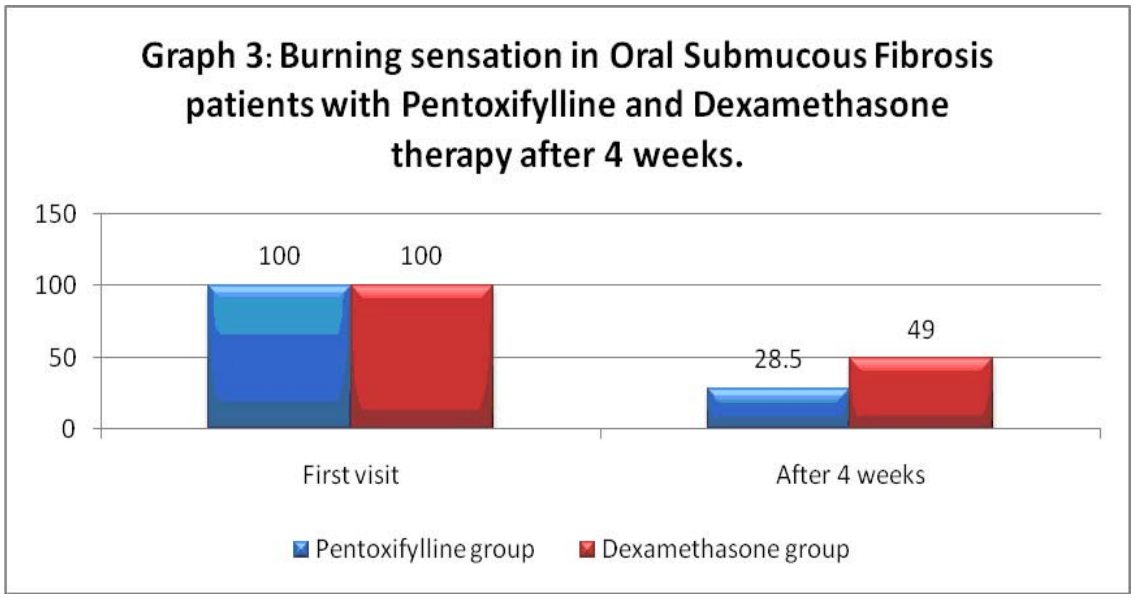
Table 2 : Distribution of Oral Submucous Fibrosis patients in Pentoxifylline and Dexamethasone Group according to Classification of OSF proposed by Ranganathan K et al.

Clinical classification of OSF	Pentoxifylline Group	Dexamethasone Group
Group I	0	0
Group II	12	17
Group III	8	3
Group IV	0	0

Clinically, all the patients in both the groups were classified according to the classification proposed by Ranganathan K et al. There were 12 patients in group II and 8 patients in group III in the Pentoxifylline group. There were 17 patients in group II and 3 patients in group III in the Dexamethasone group. The particulars are given in table no.2 and graph no.2.

The major presenting complaint among all patients in both the groups was burning sensation in the mouth on eating spicy food and reduced mouth opening. Other features like decreased tongue protrusion, and loss of cheek flexibility were noted in all the patients and were taken as criteria of the study.

Adverse effects as specified in the literature were at long treatment period. In the present study of 12 weeks of treatment, no significant adverse effects were noted, except for transient pain locally when Dexamethasone with Hyaluronidase was given intralesionally which subsided after few minutes.

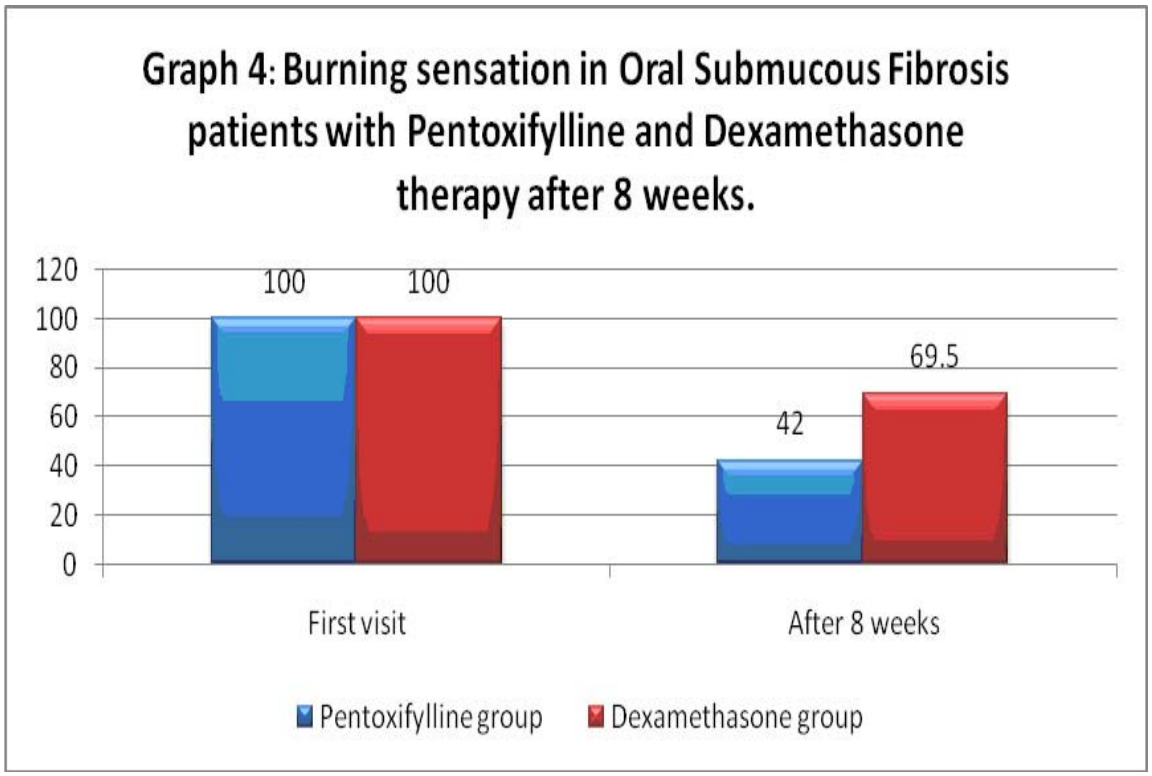


The particulars are given in table no 3 and graph no 3.

Table 3 : Burning sensation in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 4 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of patients	Mean	SD	Mean	SD	
First visit	20	100.00	0.00	100.00	0.00	-
After 4 weeks	20	28.50	8.13	49.00	8.52	<0.001

In the present study, all the patients in both the groups showed reduction in burning sensation. The mean reduction in burning sensation in the pentoxifylline group at the end of 4 weeks compared to the first visit was 28.5%. The minimum decrease in burning sensation was 10 % in two patients and maximum decrease in burning sensation was 40 % in three patients. The mean reduction in burning sensation in the Dexamethasone group at the end of 4 weeks compared to the first visit was 49 %. The minimum decrease in burning sensation was 40 % in eight patients and maximum decrease in burning sensation was 60 % in six patients. There was significant reduction in the burning sensation at the end of 4 weeks in dexamethasone group compared to pentoxifylline group ($P < 0.001$). The particulars are given in table no 3 and graph no 3.

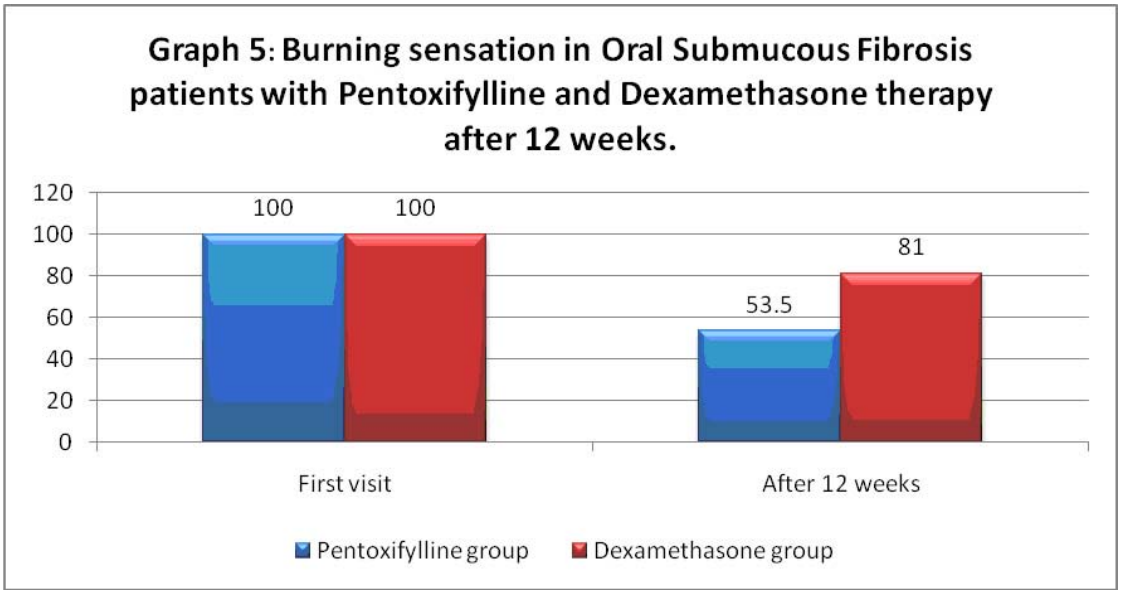


The particulars are given in table no 4 and graph no 4.

Table 4 : Burning sensation in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 8 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of patients	Mean	SD	Mean	SD	
First visit	20	100.00	0.00	100.00	0.00	-
After 8 weeks	20	42.00	8.94	69.50	8.87	<0.001

In the present study, the mean reduction in burning sensation in the pentoxifylline group at the end of 8 weeks compared to the first visit was 42 %. The minimum decrease in burning sensation was 20 % in one patient and maximum decrease in burning sensation was 60 % in one patient. The mean reduction in burning sensation in the Dexamethasone group at the end of 8 weeks compared to the first visit was 69.5 %. The minimum decrease in burning sensation was 50 % in one patients and maximum decrease in burning sensation was 80 % in six patients. There was significant reduction in the burning sensation at the end of 8 weeks in dexamethasone group compared to pentoxifylline group ($P < 0.001$). The particulars are given in table no 4 and graph no 4.

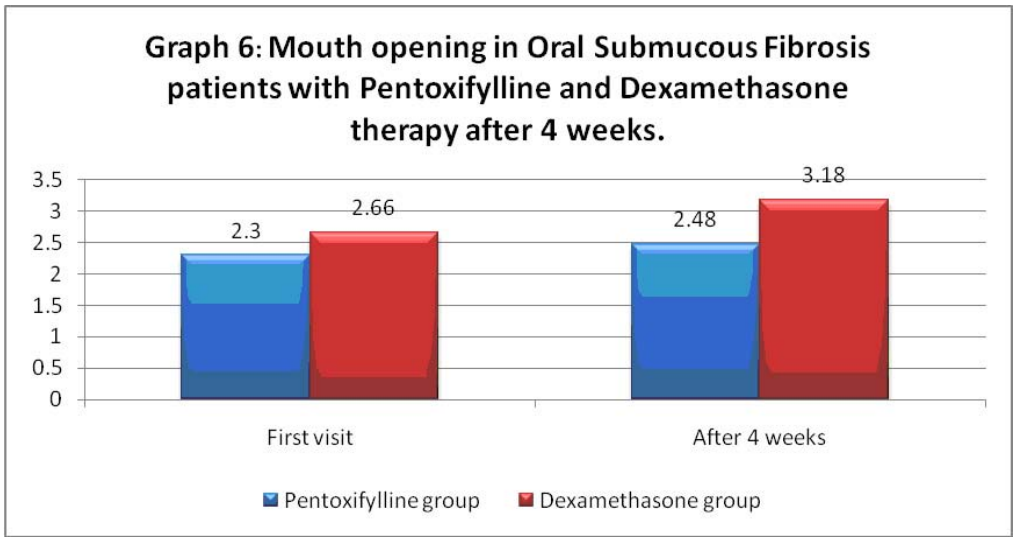


The particulars are given in table no 5 and graph no 5.

Table 5 : Burning sensation in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 12 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of patients	Mean	SD	Mean	SD	
First visit	20	100.00	0.00	100.00	0.00	-
After 12 weeks	20	53.50	8.75	81.00	6.41	<0.001

In the present study, the mean reduction in burning sensation in the pentoxifylline group at the end of 12 weeks compared to the first visit was 53.50 %. The minimum decrease in burning sensation was 30 % in one patient and maximum decrease in burning sensation was 60 % in eleven patients. The mean reduction in burning sensation in the Dexamethasone group at the end of 12 weeks compared to the first visit was 81 %. The minimum decrease in burning sensation was 70 % in three patients and maximum decrease in burning sensation was 90 % in five patients. There was significant reduction in the burning sensation at the end of 12 weeks in dexamethasone group compared to pentoxifylline group ($P < 0.001$). The particulars are given in table no 5 and graph no 5.

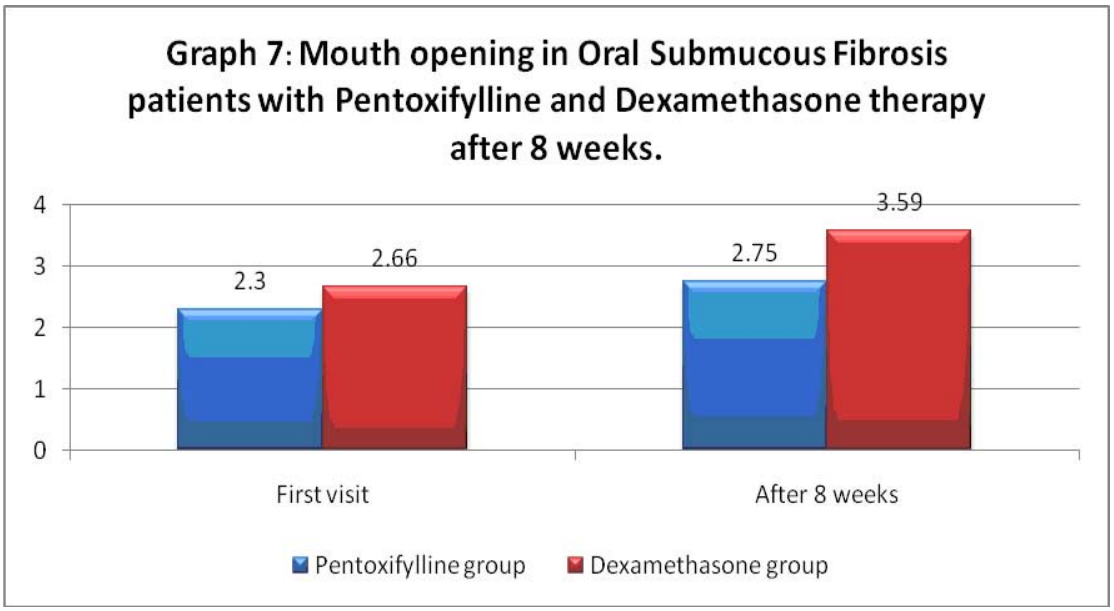


The particulars are given in table no 6 and graph no 6.

Table 6 : Mouth opening in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 4 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	2.30	0.61	2.66	0.52	0.048
After 4 weeks	20	2.48	0.65	3.18	0.60	0.001

In the present study, all the patients in both the groups showed improvement in mouth opening. The mean improvement in mouth opening in the pentoxifylline group at the end of 4 weeks compared to the first visit was 0.18 cm. Two patients showed no improvement in mouth opening and in one patient, maximum improvement was 0.4 cm. The mean improvement in mouth opening in the Dexamethasone group at the end of 4 weeks was 0.52 cm. The minimum improvement in mouth opening was 0.3 cm and maximum improvement was 0.9 cm. There was significant improvement in mouth opening at the end of 4 weeks in dexamethasone group compared to pentoxifylline group. (P = 0.001). The particulars are given in table no 6 and graph no 6.

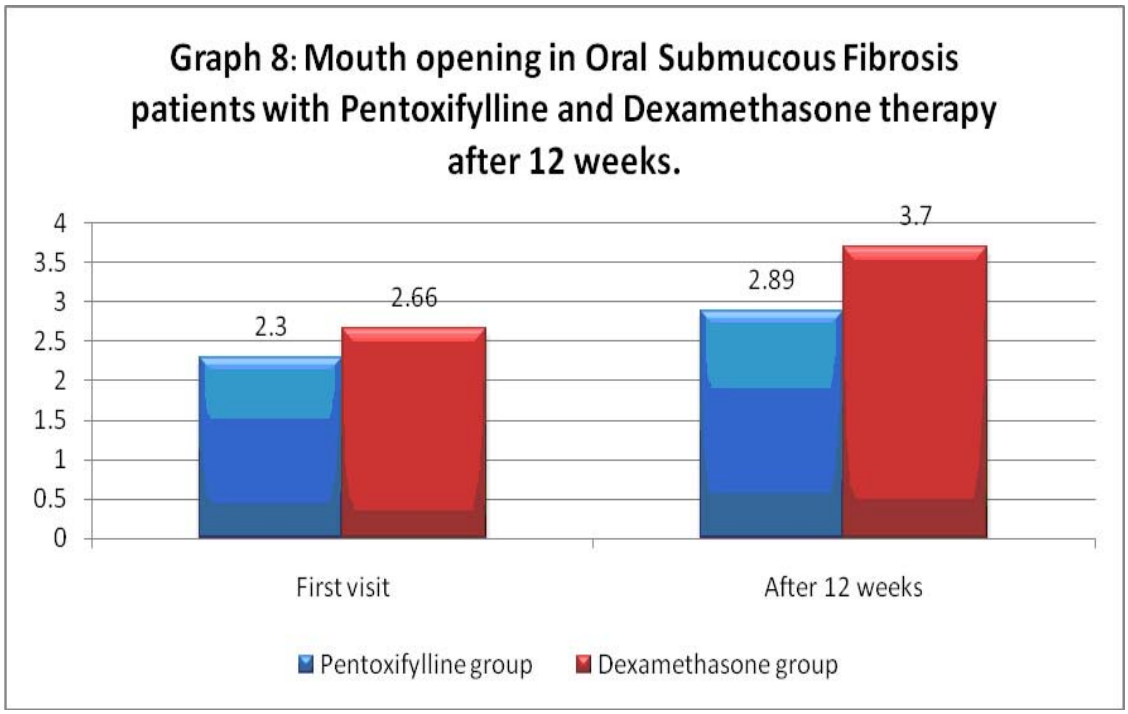


The particulars are given in table no 7 and graph no 7.

Table 7 : Mouth opening in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 8 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	2.30	0.61	2.66	0.52	0.048
After 8 weeks	20	2.75	0.69	3.59	0.57	< 0.001

The mean improvement in mouth opening in the pentoxifylline group at the end of 8 weeks compared to the first visit was 0.45 cm. The minimum improvement in mouth opening was 0.2 cm and maximum improvement was 0.7 cm. The mean improvement in mouth opening in the Dexamethasone group at the end of 8 weeks compared to the first visit was 0.93 cm. The minimum improvement in mouth opening was 0.7 cm and maximum improvement was 0.9 cm. There was significant improvement in mouth opening at the end of 8 weeks in dexamethasone group compared to pentoxifylline group ($P < 0.001$). The particulars are given in table no 7 and graph no 7.

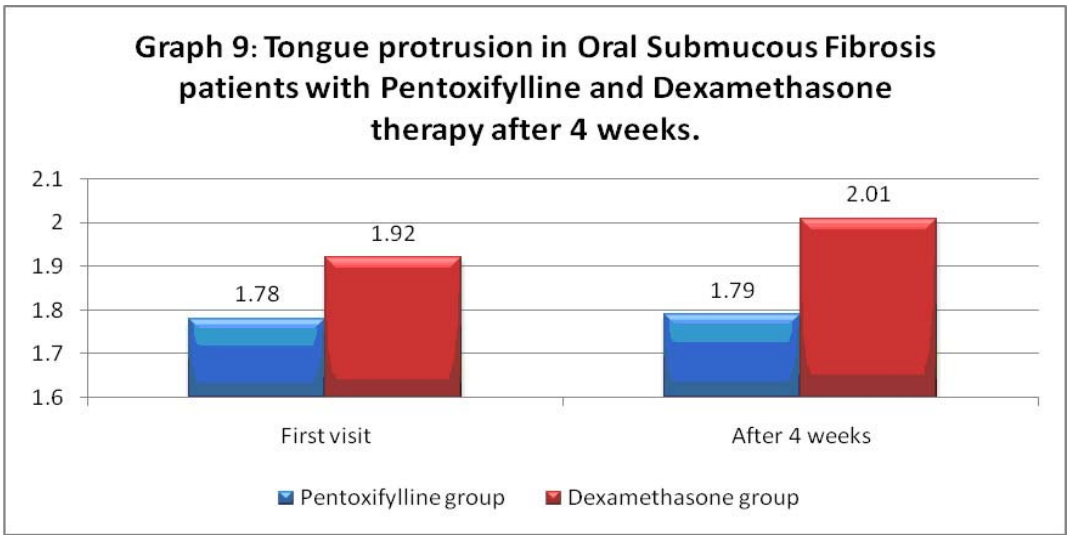


The particulars are given in table no 8 and graph no 8.

Table 8 : Mouth opening in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 12 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	2.30	0.61	2.66	0.52	0.048
After 12 weeks	20	2.89	0.65	3.70	0.52	<0.001

The mean improvement in mouth opening in the pentoxifylline group at the end of 12 weeks compared to the first visit was 0.59 cm. The minimum improvement in mouth opening was 0.2 cm and maximum improvement was 0.7 cm. The mean improvement in mouth opening in the Dexamethasone group at the end of 12 weeks compared to the first visit was 1.04 cm. The minimum improvement in mouth opening was 0.8 cm and maximum improvement was 1.5 cm. There was significant improvement in mouth opening at the end of 12 weeks in dexamethasone group compared to pentoxifylline group ($P < 0.001$). The particulars are given in table no 8 and graph no 8.

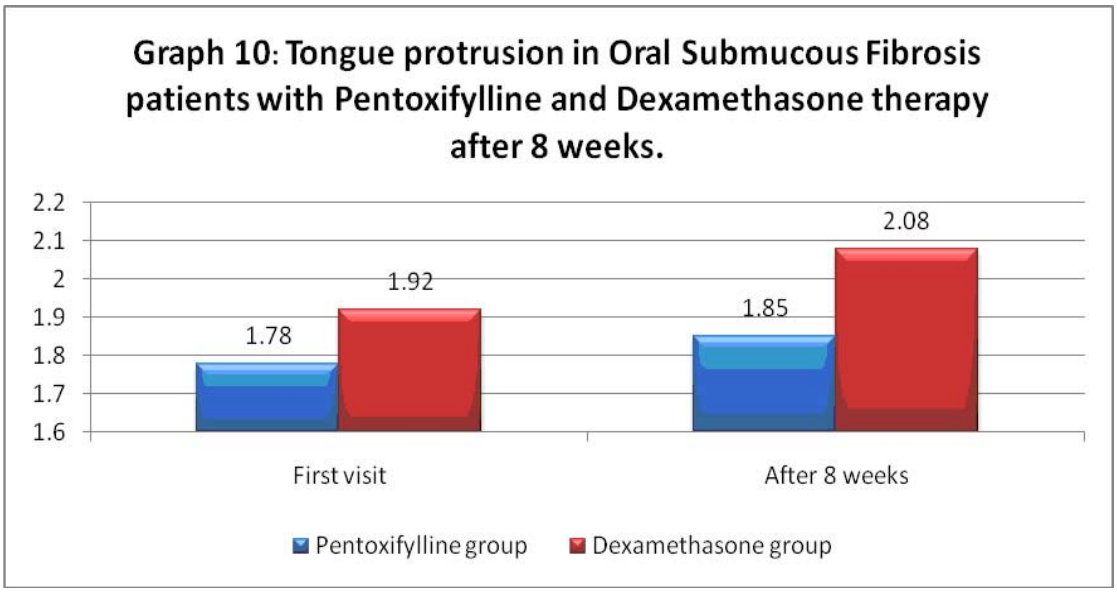


The particulars are given in table no 9 and graph no 9.

Table 9 : Tongue protrusion in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 4 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	1.78	0.66	1.92	0.44	0.418
After 4 weeks	20	1.79	0.61	2.01	0.45	0.213

In the present study, all the patients in both the groups showed improvement in tongue protrusion. The mean improvement in tongue protrusion in the pentoxifylline group at the end of 4 weeks compared to the first visit was 0.01 cm. Two patients showed decrease in tongue protrusion by 0.1 cm and in two patients, maximum increase in tongue protrusion was 0.2 cm. The mean improvement in tongue protrusion in the Dexamethasone group at the end of 4 weeks was 0.09 cm. Twelve patients showed no improvement in tongue protrusion and in one patient, maximum increase in tongue protrusion was 0.4 cm. There was no significant improvement in tongue protrusion at the end of 4 weeks in dexamethasone group compared to pentoxifylline group. (P = 0.213). The particulars are given in table no 9 and graph no 9.

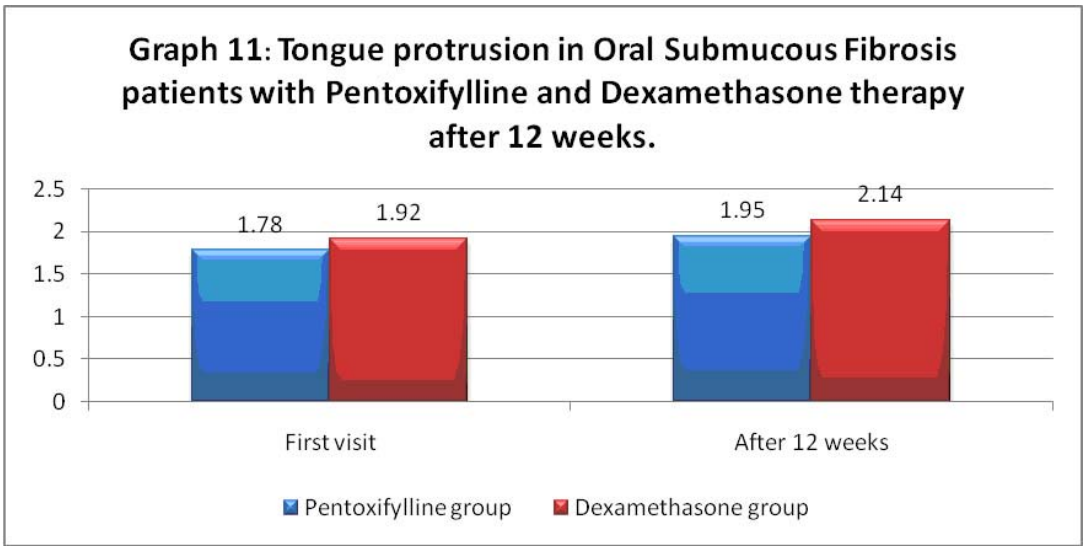


The particulars are given in table no10 and graph 10.

Table 10 : Tongue protrusion in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 8 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	1.78	0.66	1.92	0.44	0.418
After 8 weeks	20	1.85	0.59	2.08	0.43	0.157

The mean improvement in tongue protrusion in the pentoxifylline group at the end of 8 weeks compared to the first visit was 0.07 cm. Two patients showed decrease in tongue protrusion by 0.1 cm and in two patients, maximum increase in tongue protrusion was 0.3 cm. The mean improvement in tongue protrusion in the Dexamethasone group at the end of 8 weeks compared to the first visit was 0.16 cm. One patient showed no improvement in tongue protrusion and in one patient, maximum increase in tongue protrusion was 0.6 cm. There was no significant improvement in tongue protrusion at the end of 8 weeks in dexamethasone group compared to pentoxifylline group (P = 0.157). The particulars are given in table no10 and graph 10.

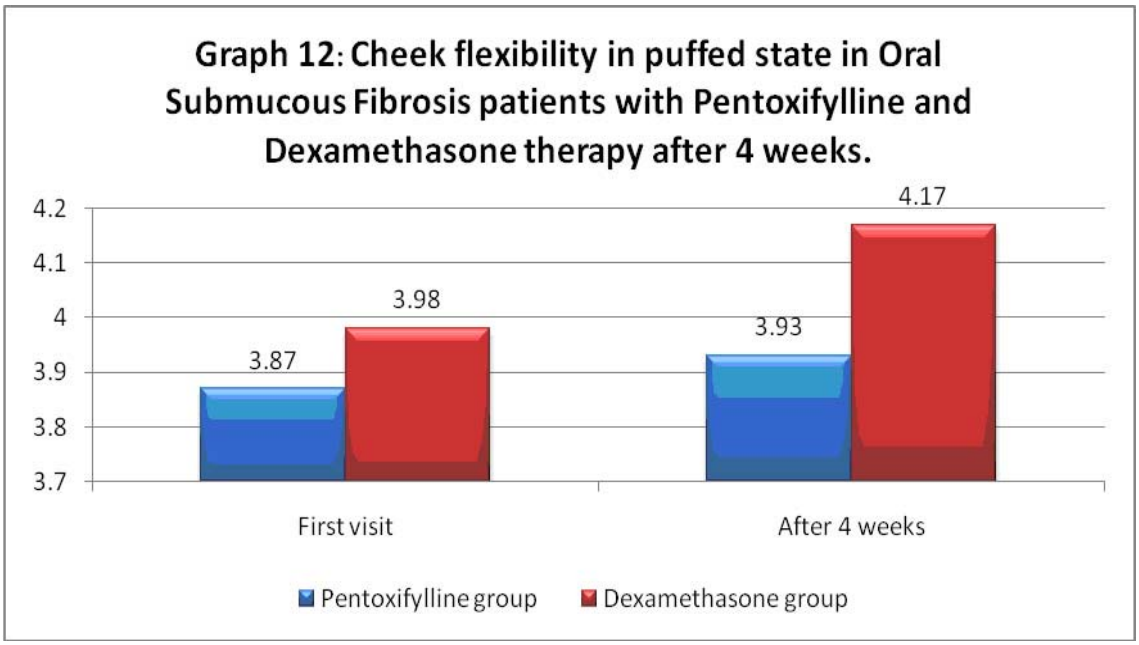


The particulars are given in table no 11 and graph no 11.

Table 11 : Tongue protrusion in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 12 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	1.78	0.66	1.92	0.44	0.418
After 12 weeks	20	1.95	0.61	2.14	0.40	0.24

The mean improvement in tongue protrusion in the pentoxifylline group at the end of 12 weeks compared to the first visit was 0.17 cm. One patient showed decrease in tongue protrusion by 0.1 cm and in one patient, maximum increase in tongue protrusion was 0.7 cm. The mean improvement in tongue protrusion in the Dexamethasone group at the end of 12 weeks compared to the first visit was 0.23 cm. Four patients showed no improvement in tongue protrusion and in one patient, maximum increase in tongue protrusion was 0.7 cm. There was no significant improvement in tongue protrusion at the end of 12 weeks in dexamethasone group compared to pentoxifylline group. (P = 0.24). The particulars are given in table no 11 and graph no 11.



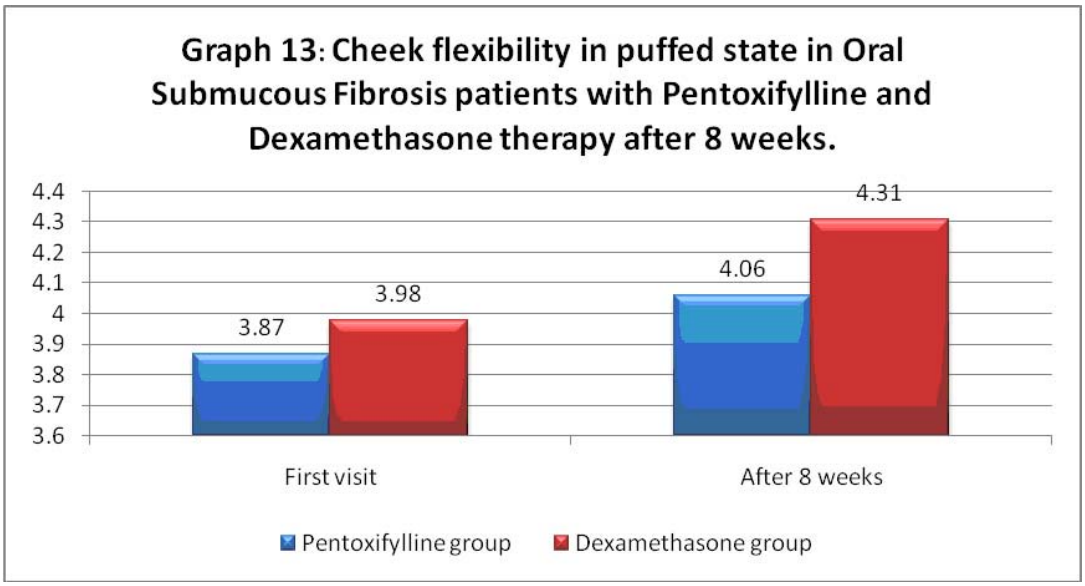
The particulars are given in table no 12 and graph no 12.

Table 12 : Cheek flexibility in puffed state in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 4 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	3.87	0.30	3.98	0.31	0.129
After 4 weeks	20	3.93	0.29	4.17	0.31	0.001

In the present study, none of the patients showed improvement in cheek flexibility in unpuffed state in both the groups.

Whereas, all the patients are in both the groups showed improvement in cheek flexibility on puffing. The mean improvement in cheek flexibility in the Pentoxifylline group at the end of 4 weeks compared to the first visit was 0.06 cm. The mean improvement in cheek flexibility in the Dexamethasone group at the end of 4 weeks was 0.19 cm. There was significant improvement in cheek flexibility at the end of 4 weeks in Dexamethasone group compared to Pentoxifylline group. (P = 0.001). The particulars are given in table no 12 and graph no 12.

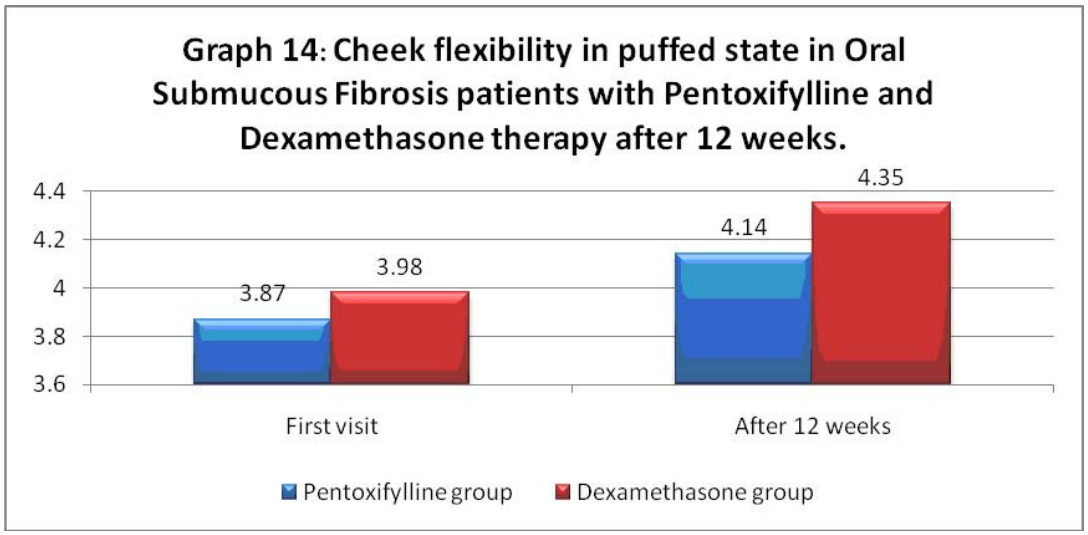


The particulars are given in table no13 and graph 13.

Table 13 : Cheek flexibility in puffed state in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 8 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	3.87	0.30	3.98	0.31	0.129
After 8 weeks	20	4.06	0.32	4.31	0.31	0.001

The mean improvement in cheek flexibility in the Pentoxifylline group at the end of 8 weeks compared to the first visit was 0.13 cm. The mean improvement in cheek flexibility in the Dexamethasone group at the end of 8 weeks compared to the first visit was 0.33 cm. There was significant improvement in tongue protrusion at the end of 8 weeks in Dexamethasone group compared to Pentoxifylline group. (P = 0.001). The particulars are given in table no13 and graph 13.



The particulars are given in table no14 and graph no 14.

Table 14 : Cheek flexibility in puffed state in Oral Submucous Fibrosis patients with Pentoxifylline and Dexamethasone therapy after 12 weeks.

		Pentoxifylline		Dexamethasone Intralesional injections		p-value
	No. of Patients	Mean	SD	Mean	SD	
First visit	20	3.87	0.30	3.98	0.31	0.129
After 12 weeks	20	4.14	0.31	4.35	0.30	0.003

The mean improvement in cheek flexibility in the Pentoxifylline group at the end of 12 weeks compared to the first visit was 0.27 cm. The mean improvement in cheek flexibility in the Dexamethasone group at the end of 12 weeks compared to the first visit was 0.37 cm. There was significant improvement in cheek flexibility at the end of 12 weeks in Dexamethasone group compared to Pentoxifylline group. (P = 0.003). The particulars are given in table no14 and graph no 14.

IV. Discussion

OSF has affected millions of individuals and is likely to reach an alarming proportion in the near future. The onset of oral sub mucous fibrosis is insidious over a period of two to five years. The patients initially complain of burning sensation in the oral cavity while consuming spicy food.

As the disease progresses the oral mucosa becomes blanched, slightly opaque and fibrous bands appear leading to difficulty in opening the mouth, inability to whistle and difficulty in swallowing.

There is constant contact between the areca nut mixture and oral mucosa, in persons with the habit of chewing areca nut. The alkaloids and flavinoids released from the areca nut get absorbed into the oral mucosa and undergo metabolism. In addition to chemical irritation from areca nut, their metabolites facilities diffusion of alkaloids and flavinoids into the sub epithelial connective tissue resulting in the juxtaepithelial inflammatory cell infiltration.[17,18]

V. Conclusion

In the present study, the increase in mouth opening, decrease in burning sensation and improvement in cheek flexibility in puffed state in oral submucous fibrosis patients showed better results by treatment with dexamethasone than pentoxifylline. Also Pentoxifylline and dexamethasone individually showed significant increase in mouth opening, decrease in burning sensation and improvement in cheek flexibility in puffed state in oral submucous fibrosis patients.

When the improvement in tongue protrusion was compared between pentoxifylline group and dexamethasone group, though dexamethasone group showed more improvement in tongue protrusion compared to pentoxifylline group, the improvement was not statistically significant. Even Pentoxifylline and dexamethasone individually did not show any significant improvement in tongue protrusion.

Nevertheless pentoxifylline did show statistically significant improvement in mouth opening, decrease in burning sensation and improvement in cheek flexibility in puffed state in oral submucous fibrosis patients. For this reason, it is concluded that Pentoxifylline is a good alternative treatment for oral submucous fibrosis in patients in whom dexamethasone is contraindicated, or those who cannot make frequent visits for intralesional injections due to disability, far reaching places or any other reason. In such oral submucous fibrosis patients it is better to substitute pentoxifylline therapy rather than not to treat at all.

Pentoxifylline can be safer and better alternative treatment for oral submucous fibrosis than dexamethasone provided local drug delivery systems are invented. Larger sample size and longer treatment duration is necessary in this regard.

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Relevance of Sex Hormones Levels With Spermogram of Infertile Men

By Thualfeqar G Mohammed, Salman A Ahmed & Majid K Hussain
Department of Biochemistry, College of Medicine, Kufa University

Abstract - Infertility is the inability of a sexually active, noncontracepting couple to achieve pregnancy in one year. The causes of male infertility include, the testicular primary failure, deficient gonadotropin secretion or due to unexplained causes. The present study was conducted to verify the relationship of male sex hormones changes with spermogram. To achieve this aim 75 infertile men with ages of 30.9±5.8 y and 35 fertile men with ages of 31.5±6.3 y (control group) were enrolled and the prevalence and pattern of endocrinological abnormalities in the patients were investigated for male infertility who attending the Central Public Health Laboratories Department of Hormones and Kamal ALSamaraie hospital period from September 2009 to April 2010.

Keywords : Azoospermia, Oligozoospermia, Teratospermia, Asthenozoospermia, FSH, LH, Prolactin, Testosterone, Infertility.

GJMR-B Classification : NLMC Code: WJ 709, WJ 752

Strictly as per the compliance and regulations of:

Relevance of Sex Hormones Levels With Spermogram of Infertile Men

Thualfeqar G Mohammed[α], Salman A Ahmed[σ] & Majid K Hussain[ρ]

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Significant ($p<0.01$) decreases were observed for the levels of total and free testosterone, and significant ($p<0.05$) increases were indicated for the levels of FSH and LH in the group of azoospermia and oligospermia when compared with the control group.

The highest levels of FSH, LH, and the lowest levels of total and free testosterone were observed in the group of azoospermia and oligospermia in comparison with other subgroups of the infertile patients.

Keywords : Azoospermia, Oligozoospermia, Teratospermia, Asthenozoospermia, FSH, LH, Prolactin, Testosterone, Infertility.

I. Introduction

The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and the testis. Gonadotropin releasing hormone (Gnrh) secreted by the hypothalamus elicits the release of gonadotrophins i.e. follicle stimulating hormone(FSH) and lutenizing hormone(LH) from the pituitary gland [1]. FSH binds with receptors in the sertoli cells and stimulates spermatogenesis. LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis [2].

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The failure of pituitary to secret FSH and LH will result in disruption of testicular function leading to infertility. Testosterone, estradiol and inhibin control the secretion of gonadotrophins through feedback mechanism [3]. Infertility is a common disorder and nearly one out of every six to eight couples suffers couples suffers from it at any given time. Infertility among couples in their respective age is more common than hypertension, diabetes, heart diseases and even the common flu [4].

Globally, it has been estimated that approximately 10-15% couples seek medical help for the problem of infertility. In 20-25% cases the problems are attributable to the male partner, while 30-40% represent female factor. In approximately 30% of cases both partners and in 15% no specific factor can be identified [5].

Male infertility can be assessed through semen analysis and hormonal profile [6]. Absence of spermatozoa in the semen ejaculate is called “azoospermia”, count less than 20 million/ml “Oligospermia”, density of 20 million/ml but motility of less than 50% is called “asthenospermia”, teratospermia is a reduced percentage of sperm with normal morphology assessed by light microscopy [7].

Male infertility is associated with a reduction in the quality of sperms. Decrease in sperm density, eventually leading to azoospermia has been found to be associated with raised FSH, LH and low testosterone level [8]. Primary hypogonadism results from disorders that affect the gonads directly, and secondary hypogonadism results from defective pituitary gonadotropin secretion.

II. Materials and Methods

Subjects : A total of 75 subjects with 35 controls, were included in the study. Subjects were categorized as azoospermia, oligozoospermia, teratospermia and asthenozoospermia on the basis of their semen concentration and motility.

Semen analysis : The seminal fluid analysis was done according to the procedure described by the World Health Organization [7].

Hormonal Assessment: Sex hormones were estimated by an enzyme immunoassay method with final fluorescent detection (ELFA), the hormones analysed

included FSH, LH, prolactin and testosterone, while free testosterone concentrations were determined by enzyme linked immunosorbent assay (ELISA).

Statistical Analysis : Data were analyzed statistically, by application of students t-test & one way ANOVA. Statistical analysis was performed with the SPSS 16 statistical Package for social sciences and also Excel 2007 with significant difference was set at $P<0.05$.

III. Results

To evaluate serum hormonal levels in various subgroups of infertile men, patients were categorized into four groups according to the results of their semen analysis. Group 1 consisted of 19 patients with azoospermia, group 2 contained 17 patients with oligospermia, group 3 comprised of 24 patients with asthenospermia, and group 4 involved 15 patients with teratospermia. The results of FSH, LH, prolactin, testosterone and free testosterone levels are shown in table 1 and Figure 1-4. Significant ($p<0.01$) decreases were observed for the levels of total and free testosterone, and significant ($p<0.05$) increases were indicated for the levels of FSH and LH in the group of azoospermia and oligospermia when compared with the control group Patients' of asthenospermia and teratospermia showed insignificant variation when compared with the control group. On the other hand prolactin levels did not show significant variation.

Table 1 : ANOVA analysis of serum hormonal profile data in subgroups of infertile men and the control group.

Parameter	A&B	A&C	A&D	A&E
FSH	0 0	0 0	NS	NS
LH	0 0	0 0	NS	NS
Prolactin	NS	NS	NS	NS
Testosterone	0 00	0 00	NS	NS
Free testosterone	0 00	0 00	NS	NS

A: Control group NO. = 35, B: Azoospermia group NO. = 19, C: Oligospermia group NO. =17, D: Asthenospermia group NO. = 24 and E: Teratospermia group NO. = 15.

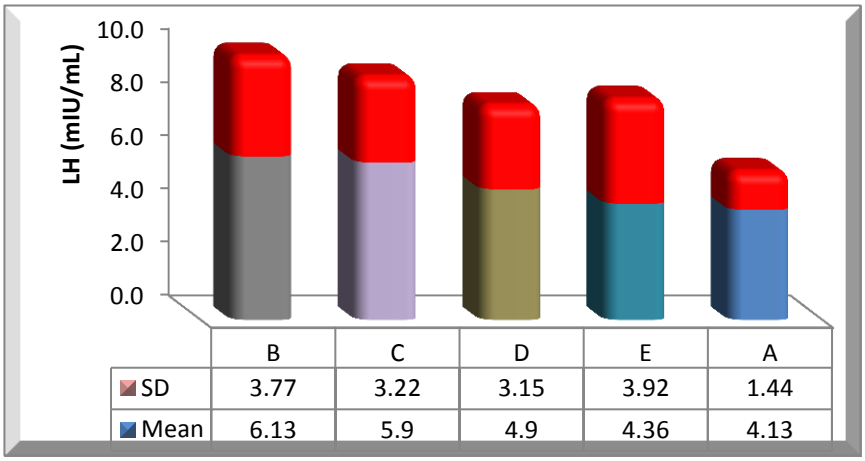

Figure 1 : Levels of serum follicle stimulating hormone (FSH) in various subgroups of infertile men and the control group.

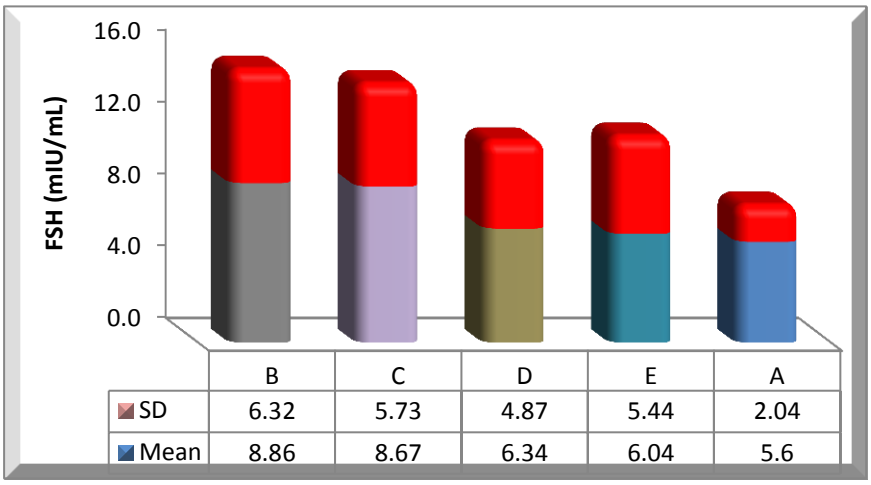

Figure 2 : Levels of serum lutenizing hormone (LH) in various subgroups of infertile men and the control group.

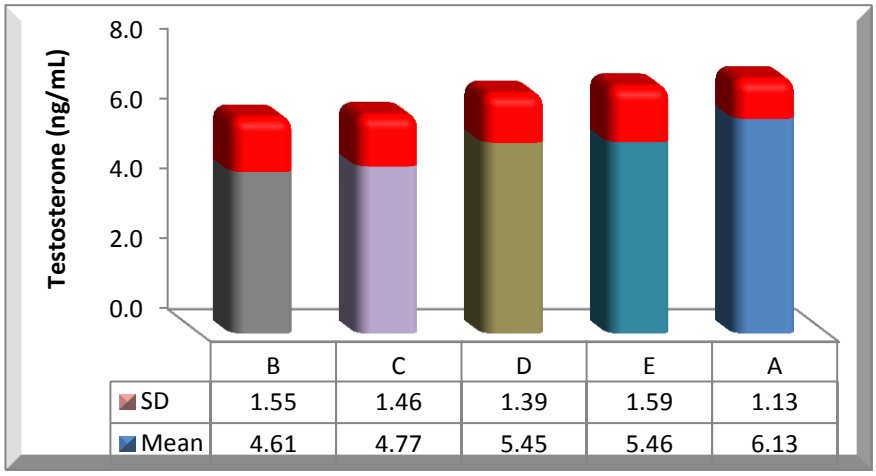

Figure 3 : Levels of serum testosterone in various subgroups of infertile men and the control group.

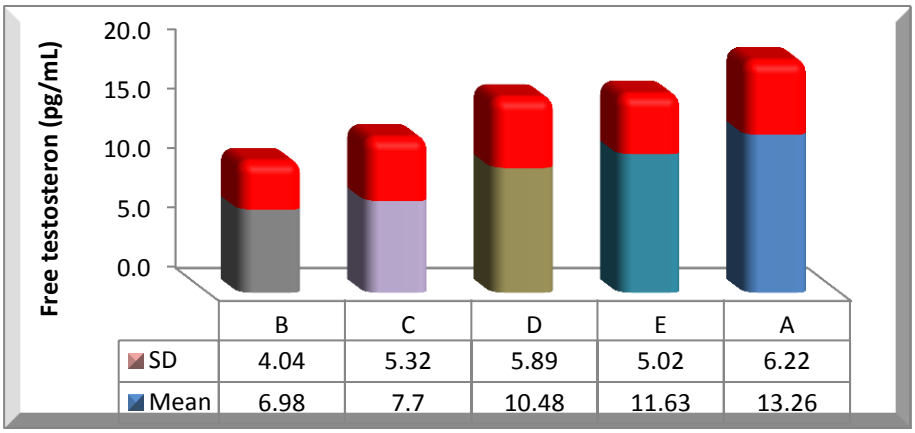

Figure 4 : Levels of serum free testosterone in various subgroups of infertile men and the control group.

IV. Discussion

FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. FSH acts directly on the seminiferous tubules whereas luteinizing hormone stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia [9].

Testosterone is essential for spermatogenesis in all species. There is some debate as to the relative levels required [10]. The androgen receptors are located on Sertoli cells [11] and the peritubular myoid cells and, since they are not expressed on germ cells, the signal must be transduced by these cells, particularly the Sertoli cells. Testosterone deficiency in men is manifested typically by symptoms of hypogonadism, including decreases in erectile function and libido [12].

The current results demonstrated elevated levels of FSH and LH with decreased levels of free and total testosterone in the azoospermia and oligospermia patients. However such difference could not be observed in patients with asthenospermia and teratospermia. These result indicated to seminiferous epithelial damage [13].

The current finding are in consistence with previous reports. Babu *et al* had reported elevated levels of FSH and LH levels with low testosterone concentration in infertile men [14]. Sulthan *et al* had illustrated elevated concentrations of FSH in infertile men due to the seminiferous epithelial destruction [15]. Similar findings had been also reported in other studies [16, 17].

V. Conclusion

These results suggested that changes of sex hormones in man are related to the alterations of spermogram. Such relationships must be considered in the management of the enrolled patients. The need for measuring prolactin levels in the evaluation of male infertility is unnecessary.

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Antiplaque Efficacy of Lemongrass Oil Mouthwash - An in-vitro Study

By Meena Anand Kukkamalla, Giliyar Subraya Bhat, Kalyan Chakravarthy Pentapati & Ruchika Goyal

Manipal University Madhav Nagar, Manipal

Abstract - Dental plaque is one of the etiologic factors in gingival and periodontal disease. Considering the uses of lemongrass oil the aim of the present study was to find out the antiplaque property in vitro. Pooled saliva was put into the tissue culture plates and incubated for 72 hours for plaque. Each of the 4 wells were treated differently with using disclosing agent, lemongrass oil mouthwash(0.25 % & 0.5%), distilled water, glodent tooth paste slurry and chlorhexidine mouthwash. The results showed that the lemongrass oil mouthwash at both the concentration showed reduction in the plaque better than that of chlorhexidine. The glodent toothpaste slurry reduced the plaue to a lesser extent to that of both lemongrass oil and chlorhexidine mouthwash. The present study concluded that the lemongrass oil mouthwash can be used as an adjunct to the mechanical oral hygiene.

Keywords : *Lemongrass oil, chlorhexidine, disclosing agent, distilled water, antiplaque, antibacterial, antibiofilm, tissue culture plate, micropipette, Lamda max.*

GJMR-L Classification : *NLMC Code: WB 350,WU 158, WU 113, WU 101.5*

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Meena Anand Kukkamalla [α], Giliyar Subraya Bhat [σ], Kalyan Chakravarthy Pentapati [ρ] & Ruchika Goyal [ω]

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Keywords : lemongrass oil, chlorhexidine, disclosing agent, distilled water, antiplaque, antibacterial, anti-biofilm, tissue culture plate, micropipette, Lamda max.

I. Introduction

Removal of dental plaque on a daily routine is one of a major factor in the prevention of caries, gingivitis and periodontitis. Dental plaque accumulation is the pre requisite for the development of gingivitis (Loe H, 1965, p-607). Gingivitis may develop into periodontitis in susceptible individuals and prevention of gingivitis is successful in prevention of periodontitis. Since both gingivitis and periodontitis are plaque associated oral conditions, the removal of dental plaque should inhibit their occurrence and progression of the disease.

Plaque control can be obtained through the mechanical removal of the biofilm by proper use of tooth brushing technique and flossing. Potential removal of supragingival bacterial plaque by means of tooth tooth brush remains the most widely accepted method of oral disease prevention. The compliance of the patient in conducting routine dental care reduces over a period of time even after education and motivation of the patients. This results in retention of plaque in interproximal surfaces of the teeth.

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Chemical control of plaque is considered to be adjunct to mechanical oral hygiene practices, the agents are most commonly used in the form of mouth rinse to prevent and control the plaque formation. Chlorhexidine digluconate is to date is a gold standard, most thoroughly studied and most effective antiplaque and anti-gingivitis agent when addressing oral hygiene (Gjermo P, 1989, p-1602). However several side effects are also associated with its use like staining of teeth and restorations, unpalatable taste with taste alteration have stimulated the search for new alternatives.

Essential oils are ideal for use in oral care products because they are both antibacterial and non-toxic – a rare combination. Lemongrass oil one of the important essential oil, extracted from Lemon grass which belongs to the section of Andropogan called Cymbopogam of the family Germineae. The botanical genus name Cymbopogon for lemongrass is derived from Greek 'cymbo' boat and 'pogon' beard. It refers to the bulbous end which is boat-shaped and the long blade-like green leaves resembling a beard.

Lemongrass has plethora of medicinal uses. It is said to have antibacterial (Pabuseenivasan S 2006, p-39), anti- inflammatory (Carbajal D, 1989, p-1983) antioxidant (Rabbani, S.I, 2005, p-28), antifungal (Taweechaisupapong S, 2012, p-37) antiseptic, astringent, analgesic, antipyretic and carminative property. Because the herb has not been studied extensively, its effectiveness is based mainly on its centuries-old reputation as a folk remedy. Considering the various uses of lemongrass oil an attempt is being made to harness the properties, use of lemongrass oil as a mouth rinse was planned for its antiplaque property. The aim of the present study was to evaluate the efficacy of lemongrass oil mouth-rinse as a chemical plaque control agent in- vitro, by assessing the reduction in the plaque and comparing lemongrass oil mouth wash with chlorhexidine mouth wash, glodent tooth paste and distilled water.

II. Materials and Methods

The present work was an in-vitro study in which the materials used were tissue culture plate (6x4 wells), micropipette; set at 10ml, micropipette tips, pooled saliva from the volunteers, lemongrass oil mouthwash 0.5% and 0.25%, chlorhexidine mouthwash, glodent

toothpaste slurry, erythrosine disclosing agent, ELx 800Ms (ELISA reader).

Tissue culture plate was taken and in each well 10 ml of the pooled saliva was added and was kept in incubator for 72 hours at 37° C, which is equivalent to the temperature of the oral cavity. After 72 hours the saliva present in the wells of the tissue culture plates are removed from the wells of the tissue culture plates which left behind the plaque that was formed at the base and around each well.

1st row of wells (4 wells) was taken as control in which only disclosing agent was added and after 30 seconds it was rinsed with distilled water with the help of micropipette. In the second row of wells lemongrass oil mouthwash (0.5%) was added, kept for 30 seconds and was pipetted out. Later one drop of disclosing agent was added, kept for 30 seconds after which it was rinsed with the distilled water with the help of micropipette. Likewise in the third row lemongrass oil (0.25%), fourth row distilled water, fifth row glodent toothpaste slurry, and sixth row chlorhexidine 0.2% was added and same procedure was repeated as it was followed in the second row of wells in tissue culture plate. After using all the different agents 10ml of distilled water was added in all the wells and was kept in the ELx 800Ms machine for the analysis. The ELx 800Ms was set at 540nanometer as the absorbency range of erythrosine was 525- 530nanometer. The readings were obtained by the printer connected to the machine. The results were tabulated using the One way analysis of variance (ANOVA) followed by post-hoc Tukey's test.

	N	Mean	SD	p-value	Post-hoc test
1	4	.5715	.10741		
2	4	.3755	.04429		
3	4	.3858	.08213	0.003	1>2,3
4	4	.5635	.06457		4>2,3
5	4	.4450	.07875		
6	4	.4302	.03642		

Table 1 : Comparative evaluation of lemongrass oil 0.5%, 0.25%, chlorhexidine mouthwash, glodent toothpaste slurry and distilled water.

Sample	Disclosing solution	LGO mouth wash 0.5%	LGO mouth wash 0.25%	Distilled water	Glodent tooth paste slury	Chlorhexidine mouthwash
1	0.557	0.410	0.495	0.526	0.447	0.427
2	0.567	0.379	0.323	0.542	0.481	0.442
3	0.705	0.401	0.403	0.653	0.418	0.412
4	0.592	0.312	0.322	0.508	0.395	0.383
Total	2.421	1.502	1.543	2.229	1.741	1.664
Mean	0.60525	0.3755	0.38575	0.55725	0.43525	0.416

Table 2 : Readings obtained: ELISA Reader (ELx800MS) → 540nm.

III. Results

Multiple comparisons were performed using One way analysis of variance (ANOVA) followed by post-hoc Tukey's test. Overall there was a significant difference in the mean scores between the groups ($p=0.003$). Post hoc analysis showed that group 1 had significantly higher mean than group 2 and 3. Similarly group 4 had significantly higher mean than group 2 and 3. Optical density due to the addition of disclosing agent was more for group 1 and 4 than group 2 and 3 implies that the group 2 and 3 had significantly less amount of plaque than group 1 and 4. There were no significant differences between Group 6 and other materials.

IV. Discussion

Dental plaque is a biofilm adhering to the tooth surface or other hard surfaces in the oral cavity including removable and fixed restoration. It can be readily visualized on teeth after 1 – 2 days with no oral hygiene. Plaque is whitish, grayish / yellow and has globular appearance. Plaque is typically observed on the gingival 3rd of the tooth surface (Newman, 2005, p-98). A common method of detecting the plaque is by the use of disclosing agent. They are available in tablet, lozenges or wafers, which contain dye or other colouring agents. The various available disclosing agents are erythrosine (PLAKSEE), two tone dye (Alpha Plaque), PLAKLITE, Skinners iodine, Mercurochrome solution (0.5%), Bismark brown (Easlick disclosing solution) and Malachite green (Wilkins EM , 1983, p-405), (Woodal, IR, 199, p-288).

The disclosing solution chosen for the study was erythrosine as it had a single wavelength, which can be easily measured by using the ELISA reader. Erythrosine is a highly coloured molecule that absorbs light near 500nm and emits longer wavelength. The λ max of erythrosine was 525nm, as UV spectrum of erythrosine showed maximum absorbance at 529nm (Ramakrishnan SP, 2007, p-361). In another study by Tinsley D and RG Chadwich (1997) said that the λ max of erythrosine was 530 nm (Tinsley D, 1997, p-67). Based on the above studies the wavelength in the present study was set at 540nm.

The interaction between saliva-coated tooth surfaces and pathogenic bacteria is partly governed by electrostatic and hydrophobic interactions, providing a solid rationale for using chemical agents as part of a plaque-control routine (Rosin M, 2002, p-392). Removal of dental plaque on a regular basis and prevention of its accumulation on teeth is the critical component of regular oral care. Even though the mechanical plaque removal remains the primary method used to maintain oral health; an improved understanding of the infectious nature of the dental disease has revitalized the interest in chemical methods of plaque control. Mouth washes containing essential oils are used for many years in the prevention and treatment of periodontal disease. Recent studies have demonstrated that essential oil mouth washes was effective as chlorhexidine mouthwash in inhibiting the plaque regrowth (Rosin M, 2002, p-392), (Riep BG, 1999, p-164) as they can penetrate the plaque biofilm, kill the pathogenic micro-organisms by disrupting their cell wall and inhibit their enzymatic

activity (Ouhayoun JP, 2003, p-10). Essential oil mouth wash prevent bacterial aggregation, slows their multiplication and extract the bacterial endotoxins (Seymour R, 2003, p-10). The mechanisms by which essential oils can inhibit microorganisms may be due to their hydrophobicity, due to which they get partitioned into the lipid bilayer of the cell membrane, rendering it more permeable, leading to leakage of vital cell contents (Burt S, 2004, p-223), (Juven J, 1994, p-626), (Kim J, 1995, p-2839). Impairment of bacterial enzyme systems may also be a potential mechanism of action (Wendakoon C, 1995, p-280). This suggests that an effective mouthwash must also penetrate the plaque biofilm.

In the present study lemongrass oil has shown be an effective antiplaque agent at both 0.5% and at 0.25% in the mouth wash which was more effective than that of the chlorhexidine. The glodent toothpaste slurry had also reduced the plaque but to a lesser extent than both the lemongrass oil 0.25% and 0.5% and chlorhexidine mouth wash. (Table 1 and 2) The present study can be related to the study done by S. Taweechaisupapong et al 2012, where they stated that exposure of Candida cells to subcidal concentration of lemongrass oil can reduce the adherence ability of the cells in inhibitory effect on biofilm formation (Taweechaisupapong S, 2012, p-37). Since adherence represents a major step in biofilm formation and lemongrass oil might be used to prevent Candida biofilm associated infection (Taweechaisupapong S, 2012, p-37). The present study was done to check the anti- plaque efficacy of lemongrass oil mouthwash where the plaque is a biofilm and lemongrass oil mouth wash at both the concentrations showed decrease in the plaque. The anti-biofilm activity can be attributed to the presence of various constituents such as citral, limonene, citronellal, β-myrcene, linalool and geraniol (Rauber Cd, 2005, p-597), (Schanebtoryerg, 2002, p-1345), (Tognolini, 2006, p-1419). In the present study Chlorhexidine mouth wash also showed decrease in plaque biofilm. It has been shown that chlorhexidine binds to salivary mucins on the bacterial cell membrane, and penetrates the plaque biofilm (Ouhayoun JP, 2003, p-10). Lemongrass oil has antibacterial property and also anti-biofilm property which brings about decrease in the bacterial load and inhibits plaque biofilm formation. Based on this above property, lemongrass oil mouthwash can be used as adjunct to mechanical plaque control in the prevention of gingival and periodontal disease.

V. Conclusion

Lemongrass oil mouthwash at both 0.25% and 0.5% was effective in reduction of the plaque. Based on this property, lemongrass oil mouthwash can be used as adjunct to mechanical plaque control in the prevention of gingival and periodontal disease.

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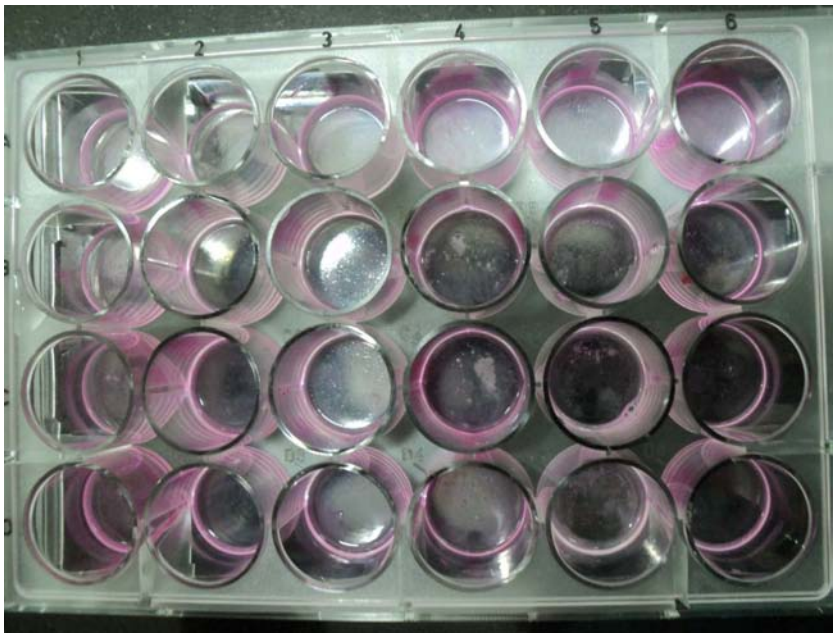

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Sustainability of HIV/AIDS Care & Support Programmes

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Acknowledgement - Igratefully acknowledge Prof. Yemane Berhane, program and theory review course instructor as well as advisor, for his professional support and advice in the whole process of developing this review. At this junction, it is a must for me to give special thanks to all my MPH course instructors for their support, motivation, and regular follow up during my stay at ACIPH. Finally, I own a debt of gratitude to all my family members for their unreserved support and encouragement to make a difference in my life.

GJMR-J Classification : *NLMC Code: WY 153.5,WD 308, WC 142, WC 144*

Strictly as per the compliance and regulations of:

Sustainability of HIV/AIDS Care & Support Programmes

Berhanu Abebe

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II. Summary

For the past three decades, the human immunodeficiency virus (HIV) infection has spread to every corner of the world. It has killed more than 25 million people since 1981 and more than 30 million people (22 million in sub-Saharan Africa alone) are now infected with HIV, which causes AIDS. Such impact alert international donor agencies to increase resources tremendously to reach significant proportion of people by creating access to basic care and prevention programs in countries worst hammered by the epidemic. Universal access to prevention and treatment for all is an integral part of the global agenda to mitigate the HIV pandemic. However, major challenges exist in combating the current HIV infection with regard to access to treatment, efficiency, quality, and sustainability of existing programs.

Sustainability of health programmes and services can be defined as the capacity to maintain programme services at a level that will provide ongoing prevention and treatment for a health problem after of HIV, various local and international organizations are exerting efforts through different systems and approaches towards the prevention and termination of major financial, managerial and technological assistance from an external donor.

The issue of sustainability gets an international agenda since its advent in the 1980s. From the time of its advent, the question of sustainability is always a challenge for health care organization particularly in developing countries. Understanding the essence of sustainability requires analyzing its four elements: technical, programmatic, social and financial sustainability. Sustainability measurement relies on the intended targeted/intervention change that happened at individual level, organization level or both. Implementing change at one level while taking into consideration the context of the others (e.g., individual versus group, facility, or system), will produce the most long-lasting impact.

Addressing the sustainability issue of an HIV care and support intervention is a dilemma. Some approaches such as community based prevention and rehabilitation, community based investment, participation at grass root level, providing resources, and trainings are helpful in establishing and formalizing long-term sustainability. Provision of necessary care and supports to the expectation level of the needy in an equitable manner are good characteristics of public healthcare and risk reduction. But there are times, where by the technical (ideologies, knowledge etc) and non-technical (funds, infrastructure etc) determinants gets impaired by the global, national and regional factors from maintaining the equity.

III. Background

For the past three decades, the human immunodeficiency virus (HIV) infection has spread to every corner of the world. It has killed more than 25 million people since 1981 and more than 30 million people (22 million in sub-Saharan Africa alone) are now infected with HIV, which causes AIDS(1). Although the overall percentage of HIV prevalence has stabilized, the number of people with the infection has gradually increased for the fact that new infection cases are occurring every year and the treatments give additional life for the HIV infected people(2).

In response to the occurrence control of HIV/AIDS with the view of making a sustainable change(3). Care and support is one of the focus areas that call attention of these interest groups. The provision of proper care and support for PLWHA and for their families can contribute in prolonging

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healthy lives(4). However, the implementation of those programs is hindered by challenges at one time or another and at different level. Many of the innovations which demonstrated success during project launch are eventually end up in failing to show achievements for the targets as well as for the implementers. Achieving Success on some projects while others are failing is a question for investigators(4). Besides success, the question of sustainability is always a challenge for health care organization particularly in developing countries (5-8).

Sustainability of health programmes and services can be defined as the capacity to maintain programme services at a level that will provide ongoing prevention and treatment for a health problem after termination of major financial, managerial and technological assistance from an external donor. To ensure the sustainability of HIV care and support programmes, strategies must be built into project design and implementation to enable HIV efforts to continue long after donor-supported projects are completed(9). This is particularly important in developing countries which are highly dependent on external funding sources(9). .Hence, it is worthy to plan and implement the donor-funded programs to the highest level to ensure sustainability(10). community-based and integrated approaches help to foster the best use of resources in the provision of care and support for PLWHA(10). However, due consideration has not been given to the sustainability aspect(5). This literature review explores sustainability of HIV/AIDS care and support programs with emphasis in developing countries.

IV. Objectives of The Review

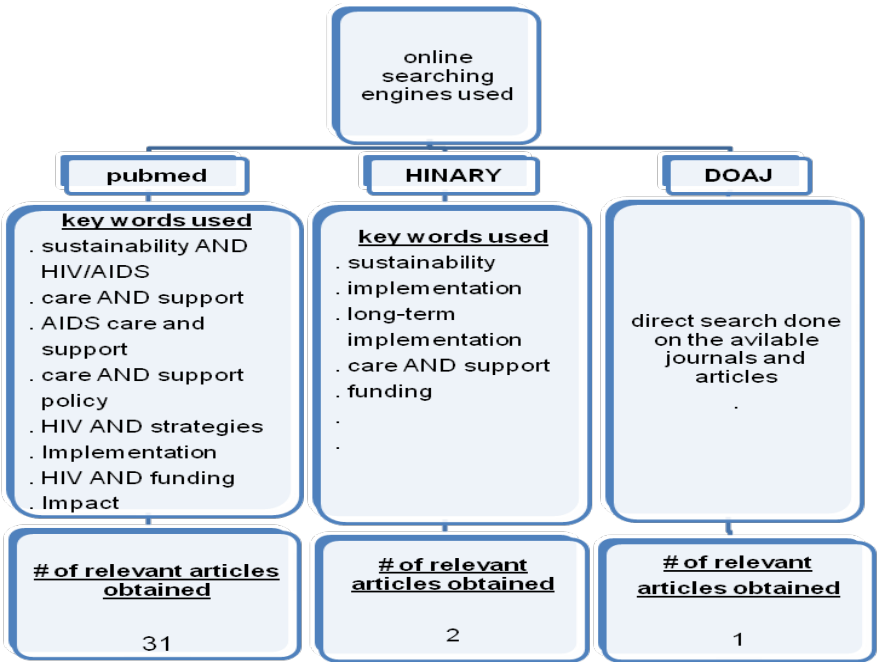
General objective:

- to understand and explain the sustainability of HIV/AIDS care and support programs.

Specific objectives:

- to figure out basic and associated determinants that are affecting sustainability of HIV/AIDS care and support programmes in developing countries.
- to develop and recommend a frame work that can be used to sustain health projects.

V. Methods



Total # of articles used as key references = 34.

Figure 1 : Search strategy and key words used.

Search criteria's :

- articles published with English language.
- articles published in the last 10 years.
- various combinations of key words within specific searching engine.
- repeated articles are dropped.

VI. Synthesis

Over the past three decades HIV/AIDS is affecting the world human development, Africa is taking the lion share of the burden. The shocking impacts are indicated on the health and demographic indicators (life expectancy at birth e.g. life expectancy at birth in Botswana fell from 65 years in 1990 to less than 40 years by 2005(11), healthcare assistance, age and sex distribution), economic indicators (income, work force, and economic growth), Social indicators (education and knowledge), and other indicators (governance, gender inequality and human rights)(12). Such impact alert international donor agencies to increase resources tremendously to reach significant proportion of people by creating access to basic care and prevention programs in countries worst hammered by the epidemic(6). Universal access to prevention and treatment for all is also an integral part of the global agenda to mitigate the HIV pandemic(13) However, major challenges exist in combating the current HIV infection with regard to access to treatment, efficiency, quality, and sustainability of existing programs(6, 7).

a) Overview of sustainability

The issue of sustainability gets an international agenda since its advent in the 1980s(14). Understanding the essence of sustainability requires analyzing its four elements: technical, programmatic, social and financial sustainability. The Technical sustainability refers the continuous availability of high-quality, facility-based HIV clinical services aligned with national standards (Skilled professionals, adequate laboratory, pharmacy infrastructure, sufficient equipment and commodities). Programmatic sustainability refers effective management, coordination and implementation of facility-based HIV services (robust logistics; commodity and supply management systems; functional communications). Social sustainability refers to sustained HIV activities, which rely on continued demand for HIV services by communities (acceptability, accessibility, affordability and culturally sensitive). Financial sustainability refers the presence of adequate and continuous funding to achieve HIV service targets and objectives. This is a major challenge in resource-limited countries(13, 15).

Usually, sustainability measurement relies on the intended targeted/intervention change that happened at individual level, organization level or both. Implementing change at one level while taking into consideration the context of the others (e.g., individual versus group, facility, or system), will produce the most long-lasting impact(16). These factors are likely also to be important in work aimed at sustaining organizational innovations that have been successfully introduced. Some factors (e.g., a supportive organizational culture) are likely to come into play earlier on in the introduction of an organizational innovation, whereas others are likely to be more important in sustaining, maintaining, and routinising change(17). Bringing desirable change in individual vise-a-vise organizational performance are two different tasks that require not only different instruments for measuring changes, but acquisition of in-depth knowledge of the processes that control adoption or assimilation of the innovation at either level(16).

b) Challenges of PLWHA

People living with HIV/AIDS face tremendous challenges, including mental health, lack of care and support, stigma-a dynamic process of devaluation that 'significantly discredits' an individual in the eyes of others(18), and depression(19). Though projects and interventions like HIV therapy and care programs are designed and remains working in the fight against HIV in developing countries, it is not touching the ground as per the wishes. Without adequate treatment, care or support, mortality rates would continue to rise(20). PLWHA as well as family members are not only struggling with sickness, but also facing impaired productivity, declining income, and increasingly difficult choices among essentials but competing expenses such as food versus healthcare or schooling versus rent(21, 22)

c) Determinants of sustainability

HIV/AIDS care and support programs usually require two major categories of support - formal and informal. The formal social supports are those supports provided from health care and social service facilities which are established for the same or related purposes. Whereas the informal social supports are those supports originated from family, friends, and other community organizations (like churches) (23-25) that are highly recommended in managing most chronic diseases including HIV. The presence and provision of close support from family members for PLWHA promote their odds of entry into medical care(23).

In a comparative study to know the relevance of care and support among children who were placed in three domains of outcome measures (group homes, orphanages, and in kinship) those children's who were attached to the group homes performed best in almost all psychosocial variables. Consequently, children's in group homes who were receiving the necessary collaboration, care and support has demonstrated lowest level of anxiety, depression, anger, post-traumatic symptoms, disassociation and sexual concerns(26). Collaboration was found to be the basis for sustainability(27).

Provision of necessary care and supports to the expectation level of the needy in an equitable manner are good characteristics of public healthcare and risk reduction. But there are times, where by the technical (ideologies, knowledge etc) and non-technical (funds, infrastructure etc) determinants gets impaired by the global, national and regional factors from maintaining the equity(28).

Though aggressive and multidimensional strategies are designed (by foundations, donors, policy makers, and advocates) to stop the HIV infections and sustain impacts, they did not escaped from the increasing criticism for their failures to achieve the 2008 goals(29) (mid point for MDG). On contrary, counter arguments recognize that the relevance and contributions of global health sectors in mobilizing significant amount/kind of resources which was not achieved prior to HIV. The question here is how far the huge resources are contributing for the HIV/AIDS care and support program sustainably in developing world? The other critic is the global fund for HIV is the most extravagant in consuming the majority human and financial resources as compared to the measurable outcomes. Due to this, less resource is being allocated for tuberculosis (TB), malaria, and malnutrition. This leads the vulnerable groups in to further complication and public health problems. Most often, small rural, African villages are the most vulnerable from such negligence. In situations where by the HIV/AIDS case is rampant vise-a-vise low service coverage for the care and support interventions, the poor PLWHA are still challenged with the HIV infection consequences. In order to address these challenges horizontal integration, family wellness, evidence based prevention, and applications of highly active and vibrant systems are advised(29, 30)

In a research conducted to identify the decision making process for HIV/AIDS resource allocation including for care and support in sustainable ways, it was revealed that the resource allocation begins with the selection of HIV/AIDS programmes and with available data. This is followed by the funding level and the level of experiences they acquired for each programmes(31). In the process of allocating the resources, external individuals, other organizations, and other intangible factors have an important influence either in supporting or refusing the ultimate decision. This by itself either benefits or hinders the HIV/AIDS care and support programs in developing countries. On a similar research, the type of tools or frameworks that are used by decision makers in allocating resources for HIV/AIDS were analyzed and figured out that for small organization or local level decisions the use of such formal techniques is not common. However, for a national level organization, they use rational economic models to analyze the epidemiological and the cost-effectiveness. However, the use of other operations research techniques or framework is not common(31).

On the same token in another study the private sector resources mobilization, efficiency in disbursing the funds, and assurances are not satisfying the expectations of the people living with HIV/AIDS, the participating stakeholders, and other multilateral organizations in sub-Saharan Africa(32), a challenge for the sustainability of HIV projects.

d) Sustainability strategies

Due to the escalating number of HIV/AIDS infection in developing countries, there is a high demand for system-level interventions. This is a promising approach aims at improving the proper functioning of the organization as well as the delivery of services to the community in coordinated manner. System-level interventions are a promising approach to HIV/AIDS prevention because they focus on (a) evidence based HIV prevention and care programs (b) develop and establish policies and procedures that maximize the sustainability of on-going prevention and care efforts (c) improve the decision making processes such as incorporating the needs of communities into their tailored services(33).

Addressing the sustainability issue of an HIV care and support intervention is a dilemma. Some approaches such as CBPR, community based investment, participation at grass root level, providing resources, and trainings are helpful in establishing and formalizing long-term sustainability(8). But, most of the organization in developing countries, rather than focusing on the mentioned approaches, they were just concentrating on the provision of food aid within their HIV/AIDS care and support programmes with a rationale that PLWHA are not food secured. Food supplementation, however, was quickly recognized as an unsustainable and incomplete intervention(34). A growing body of research suggests that community readiness to adopt and implement evidence-based interventions is essential for sustainability(15).

Even though the expected outcome towards care and support program is to the minimum level due to the mentioned reasons, it was argued that 'care agenda' needs top priority and urgency by the international health policy in its framework, strategies and actions. Furthermore, it stresses that other non health sector should support and strengthen the policy as well as the community home-based care to the broader sense to ensure sustainability(10).

Caring for a person with HIV/AIDS requires considerable time and other resources, which is compounded in many developing countries(10). In response to the growing need for a more programmatic

approach to care for persons living with HIV/AIDS, the World Health Organization (WHO), in consultation with a wide group of experts, developed a framework for 'Comprehensive Care across a Continuum' later known simply as the 'Care Continuum' (WHO 2000b). The intent of the model was to promote, create and sustain a 'holistic' approach to care and support for persons living with HIV/AIDS(10). This approach is believed to be an important advance for the fact that it illustrate in creating linkage among care domains. Though this is appreciated, the 'care continuum' is criticized for poor mechanism of linking individuals with home care and peer support across the continuum. Thus the application of the model might be challenged for its intent i.e. promoting and sustaining holistic approaches to care and support for PLWHA.

VII. Limitation of The Review

The major limitation of this literature review is:

- Majority of the published articles done on sustainability are focusing about overall HIV/AIDS interventions. And identifying only the care and support from the available articles is difficult for the fact that a number of confounding factors are affecting the sustainability aspect.

VIII. Conclusions And Recommendations

HIV is still a major public health problem in developing countries. Various local and international organizations are exerting efforts through different systems and approaches towards the prevention and control of HIV/AIDS with the view of making a sustainable change. Care and support is one of the focus areas that call attention of these interest groups. However, the question of sustainability is always a challenge for health care organization.

Major factors that determine the sustainability of HIV care and support at the global, national and regional levels are; type of care and support provided, technical (ideologies, knowledge etc) and non-technical (funds, infrastructure etc). In order to address these challenges horizontal integration, family wellness, evidence based prevention, and applications of highly active and vibrant systems are advised. The following framework is also recommended based on the existing sustainability gaps,

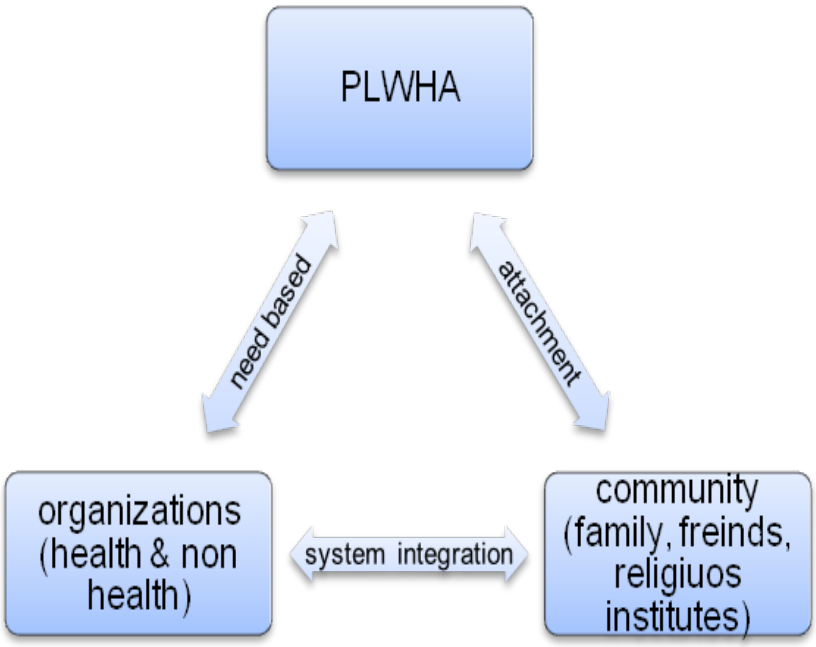


Figure 2 : Care and support for PLWHA link with organizations and community.

- PLWHA should be at the center of the any intervention, serving as a bridge in linking the organization with the community.
- Organizations (health and non health) have to give technical, programmatic, and financial support to PLWHA on need based with the view of improving quality of life.
- There has to be aggressive system integration with the existing government structure, supported with evidence based intervention.
- Community members should bear ownership and provide required support to PLWHA as well as the organizations.

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Regenerative Effect of Rat Embryonic Stem Cells Against CCl_4 Induced Liver Damage in Wister Rats

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Abstract - *Aim :* To conduct the study on regenerative effect of rat undifferentiated embryonic stem (ES) cells against carbon tetrachloride (CCl_4) induced liver damage in rats and determine their ability to differentiate into hepatocytes in the liver.

Methods : Liver injury was produced by administration of CCl_4 mixed with liquid paraffin. Liver injury induced by administration of CCl_4, 0.5 mL /kg body weight, was injected into the peritoneum of rat twice a week for 2 weeks. Control animals received an equal volume of liquid paraffin. The dose of Embryonic Stem (ES) cells for the treatment damaged liver of rats was calculated according to the cell viability count and suspension was administered through intraperitonial route, 1 x 10^5 undifferentiated ES cells (0.1 mL of 1 x 10^6cells/mL solution), genetically labeled with GFP, were transplanted into the spleens 1 d after the second injection.

Keywords : *Embryonic stem cells; Hepatic differentiation; Carbon tetrachloride.*

GJMR-G Classification : *NLMC Code: QY 60.R6, QY 140*

Strictly as per the compliance and regulations of:

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Mohammed Ibrahim [α] & Anjum A [σ]

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Methods : Liver injury was produced by administration of CCl_4 mixed with liquid paraffin. Liver injury induced by administration of CCl_4 0.5 mL /kg body weight, was injected into the peritoneum of rat twice a week for 2 weeks. Control animals received an equal volume of liquid paraffin. The dose of Embryonic Stem (ES) cells for the treatment damaged liver of rats was calculated according to the cell viability count and suspension was administered through intraperitonial route, 1 x 10^5 undifferentiated ES cells (0.1 m L of 1 x 10^6 cells/mL solution), genetically labeled with GFP, were transplanted into the spleens 1 d after the second injection. The Reparative capacity of Rat Embryonic Stem cells was analyzed in liver injured CCl4-treated male rats. Biochemical parameters including like AST and ALT, ALP and Bilirubin and total protein in serum were analyzed. The biochemical findings were supplemented with histopathological examination of rat liver sections.

Results : There was a significant increase in serum levels of AST and ALT, ALP and Bilirubin with adecrease in total protein level in the CCl_4 treatedanimals, reflecting liver injury. In the undifferentiated embryonic stem cell treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. Histological study of Es treated animals revealed normal hepatic cords without any cellular necrosis and fatty infiltration.

Conclusion : Embryonic stem (ES) cells showed significant hepatoprotective activity in rats with CCl4-induced liver damage compared with serum marker enzyme activity. Further the results are supported embryonic stem cells developed into hepatocytes-like cells with appropriate integration to form tissue.

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Keywords : Embryonic stem cells; Hepatic differentiation; Carbon tetrachloride.

I. Introduction

Liver is an important organ of body, which performs the function of detoxifying all substances, which are ingested by humans; therefore, hepatic cells are most susceptible to damage by metabolites of various allopathic drugs. These drugs cause significant hepatic damage due to formation of highly toxic metabolites. The liver aids greatly in the maintenance of metabolic homeostasis by processing dietary amino acids, carbohydrates, lipids, and vitamins; metabolizing cholesterol and toxins; producing clotting factors; and storing glycogen. Injury to the liver parenchyma associated with an influx of acute or chronic inflammatory cells is termed hepatitis. Cirrhosis refers to a progressive, diffuse, fibrotic, nodular condition that disrupts the entire normal architecture of the liver.[1, 2] Fibrosis previously was thought to be an irreversible scarring process formed in response to inflammation or direct toxic insult to the liver, but current evidence suggests that fibrosis may be reversible in some patients with chronic hepatitis B after antiretroviral therapy.[3] Liver cirrhosis is one of the most representative forms of liver fibrosis and represents a serious health problem. Recently, transplantation of bone marrow-derived cells including mesenchymal stem cells was reported to reduce carbon tetrachloride (CCl_4) -induced liver fibrosis [4-8], while fetal liver epithelial progenitor cells have also been shown to ameliorate diethyl nitrosamine-induced liver fibrosis.[9] A stem cell is an undifferentiated cell capable of renewing itself throughout its life and of generating one or more types of differentiated cells. While embryonic stem cells (ESCs) are the only ones to be totipotential, adult tissues with high cellular turnover (e.g. skin, gut mucosa and bone marrow) retain a population of stem cells with restricted differentiation potential that constantly supply the tissue with new cells.

Embryonic stem (ES) cells are self-renewing and multi-potent cells derived from the inner cell masses of preimplantation blastocysts[10, 11] , and have many

characteristics of an optimal cell source for cell replacement therapy. Theoretically, ES cells are able to be produced limitlessly, and various kinds of cell-types have been generated *in vitro* and *in vivo*. Thus, ES cells are considered to have potential to become an optimal cell Source for cell-replacement therapy.

End stage liver disease (ESLD) is the final stage of acute or chronic liver damage and is irreversibly associated with liver failure. ESLD can develop rapidly, over days or weeks (acute and sub-acute liver failure, respectively), or gradually, over months or years (chronic liver failure [12]. Currently, liver transplantation is the most effective therapy for patients with ESLD [13]. However, its potential benefits are hampered by many drawbacks, such as the relative shortage of donors, operative risk, post-transplant rejection, recidivism of the pre-existing liver disease, and high costs.

In this scenario, stem cell therapy sounds particularly attractive for its potential to support tissue regeneration requiring minimally invasive procedures with few complications. This field of research, which represents the ground from which the new discipline of "regenerative medicine" has germinated, has rapidly developed in recent years, arising great interest among scientists and physicians, and frequently appearing in newspapers headlines touting miracle cures, but arising ethical crises as well[14]. The most debated issue pertains to the use of human ESCs, as it implies, with current technologies, the destruction of human embryos. Opponents of ESC research argue that ESC research represents a slippery slope to reproductive cloning, and can fundamentally devalue human life. Contrarily, supporters argue that such research should be pursued because the resultant treatments could have significant medical potential. It is also noted that excess embryos created for in vitro fertilization could be donated with consent and used for the research [15]. The *in vitro* capability of ES cells to differentiate into hepatocytes like cells has also been proven by other investigators[16]. In general, the methods used in those studies can be divided into spontaneous and directed differentiation. For spontaneous differentiation, the formation of embryoid bodies (EBs) has been mostly utilized [17]. With directed differentiation, different processes of enrichment of a specific differentiated cell type that use elements to promote the differentiation of ES cells into an endodermal lineage, such as the addition of growth factors (GFs) and hormones[18], and the constitutive expression of hepatic transcription factors[19], have been utilized.

In the present study the regenerative effect of rat undifferentiated embryonic stem (ES) cells against carbon tetrachloride (CCl_4) induced liver damage in rats and determines their ability to differentiate into hepatocytes in the liver. Rat undifferentiated embryonic stem (ES) cells showed significant hepatoprotective activity in rats with CCl_4-induced liver damage as judged from serum marker enzyme activity. Further the results are supported as Undifferentiated ES cells developed into hepatocytes-like cells with appropriate integration into Tissue.

II. Materials and Methods

a) *Materials*

i. *Animals*

Wister rats weighing 175-200 g were obtained from the animal house of Deccan College of Medical Sciences, Hyderabad and housed in polycarbonate cages. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment with the exception of some experiments in which the animals were deprived of food, but not water, for 18-24 h before the experiments were performed. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to experimentation room and were maintained on standard conditions (23^0 c, 60-70 % relative humidity and 12 h photo period). All experimental protocols described below were approved by the ethical board.

ii. *Hepatotoxin*

a. *CCl_4 treatment*

Chemically induced hepatic injury for experimental studies should be severe enough to cause cell death or to modify hepatic functions. The mechanism of acute hepatic injury depends upon the chemical compound and the species of animals used. CCl_4 is one of the most powerful hepatotoxin in terms of severity of injury. It causes toxic necrosis leading to biochemical changes having clinical features similar to those of acute viral hepatitis [20, 21]. Liver injury was produced by administration of CCl_4 mixed with liquid paraffin. Animals were given dose of CCl_4, 0.5 mL /kg body weight, was injected into the peritoneum of rat twice a week for 2 wk throughout the experimental setup. Control animals received an equal volume of liquid paraffin.

b. Methods

Induction of estrus: if males and females are housed separately, when they are put together for mating, estrus will be induced in the female 3 days later, when the maximum number of successful mating will occur. This process enables the planned production of embryos at the appropriate time. The timing of successful mating may be determined by examining the female's vargunas each morning for a hard mucous plug. The day of detection of a vaginal plug, or the 'plug date,' is noted as day zero, and the development of the embryos is timed from this date. Full term is about 19-21. The optimal age preparing cultures from a whole

disaggregated embryo is around 13 days, when the embryo is relatively large but still contains a high proportion of undifferentiated mesenchyme, which is the main source of the culture. Most individual organs, with exception of brain and heart, begin to form at about ninth day of gestation, but are difficult to isolate until about the 11th day. Dissection is easier at 13 -14th day and most of the organs are completely formed by the 18th day. Sacrifice the mouse by cervical dislocation and swab the ventral surface liberally with 70% alcohol.

Dissect out the uteri into a 25ml or 50ml screw capped tube containing 10 or 20 ml BSS. Antibiotics may be added to BSS when there is high risk of infection. Take the intact uteri to the tissue culture laboratory and transfer to a fresh dish of sterile DBSS.

Dissect out the embryos : Tear the uterus with two pairs of sterile forceps, keeping the points of forceps close together to avoid distorting the uterus and bringing too much pressure to bear on the embryos. Free the embryos from the membranes and the placenta and place them to one side of the dish to bleed. Transfer the embryos to a fresh dish. If a large number of embryos are required, it may be helpful to place the dish on ice. [22]

i. *Enzymatic Desegregation*

Cell-cell adhesion in tissues is mediated by a variety of homotypic interacting glycopeptides some of which are calcium dependent and hence are sensitive to chelating agents such as EDTA or EGTA. Integrins, which binds to RGD motif in extracellular matrix also have calcium binding domains and are affected by calcium depletion. Intercellular matrix and basement membrane also contain other glycoprotein, such as fibronectin and laminin, which are less so, and can sometimes be degraded by glycanases, such as hyaluronidase or heparinase. The easiest approach is to proceed from a simple desegregation solution to more complex solution with trypsin alone or trysin/EDTA as starting point, adding other proteases to improve desegregation, and deleting trypsin if necessary to increase viability. In general increase in purity of an enzyme will give better control and less toxicity with increases specificity but may result in less desegregation activity.

Mechanical and enzymatic desegregation of tissues avoids problems of selection by migration and yields a higher number of cells that are more representative of the whole tissue in a shorter time. However, just a primary – explants technique selects on the basis of cell migration, dissociation techniques select cells resistant to method of desegregation and still capable of attachment.

Embryonic tissue disperses more readily and gives a higher yield of proliferating cells than those new born or adult tissue. The increasing difficulty in obtaining viable proliferating cell with increasing age is due to several factors, including the onset of differentiation, an increase in fibrous connective tissue and extracellular matrix, and a reduction of the undifferentiated proliferating cell pool. A procedure of greater severity is required to disaggregate with trypsin while still retaining viable carcinoma cells.

The choice of trypsin grade to use has always been difficult, as there are two opposing trends:

i. The purer the trypsin, the less toxic it becomes, and the more predictable its action.
ii. The cruder the trypsin, the more effective it may be, due other proteases.

In practice, a preliminary test experiment may be necessary to determine the optimum grade for viable cell yield, as the balance between sensitivity to toxic effects and desegregation ability may be difficult to predict.

Crude trypsin is by far the most common enzyme used in tissue desegregation, as it is tolerated quite well by many cells, it is effective for many cells, it is effective for tissues, and any residual activity left after washing is neutralized by the serum of the culture medium or by a trypsin inhibitor when serum free medium is used. It is important to minimize the exposure of cells to active trypsin in order to preserve maximum viability. Hence, when whole tissue is being trypsinised at 37^0 c, disassociated centrifugation and neutralized with serum in medium. Soaking the tissue for 6-18hrs in trypsin at 4^0c allows penetration with minimal tryptic activity, and digestion may then proceed for a much shorter time (20-30min) at 37^0 c. Although the cold trypsin method gives a higher yield of viable cell and requires less effort, the warm trypsin method is still used extensively.

ii. *Enzymatic desegregation by cold trypsinization*

Transfer the tissue to fresh Sterile DBSS in a $9cm^2$ Petri dish and rinse. Transfer the tissue to the second dish; dissect off unwanted tissue such as fat or necrotic material; and chop with crossed scalpels to about 3 mm cubes. Embryonic organs, if they do not exceed this size, are better left whole. a. Transfer the tissue with curved forceps to a 15-50 ml sterile centrifuge tube or universal container. Allow the pieces to settle.

Wash the tissue by re-suspending the pieces in BSS, allowing the pieces to settle and removing the supernatant fluid. Repeat this step two more times.

Remove most of the residual fluid and add 10 ml/tube/g of tissue of 0.25% trypsin at 4^0 c.

Place the mixture at 4^0C for 6-18hrs. Place the tube at 37^0 C for 20-30 min.

Add warm medium, approximately 1 ml for every 100 mg of original tissue and gently pipette the mixture up and down until the tissue is completely dispersed.

If some tissues do not disperse, then the cell suspension may be filtered through sterile muslin or stainless steel mesh (100-200 microgram), or Falcon 70 mm "cell strainer (Becton Dickinson) or the larger pieces may simply be allowed to settle. When there is a lot of tissue, increasing the volume of suspending medium to 20 ml for each gram of tissue will facilitate settling and subsequent collection of supernatant fluid. Two to three minutes should be sufficient to get rid of most of the larger pieces.

Determine the cell concentration in the suspension by hemocytometer or electronic cell counter. And check viability. The cell population will be very heterogeneous; electronic cell counting will initially require confirmation with a hemocytometer, as calibration can be difficult.

Dilute cell suspension to 1 x 10^6 per ml in growth medium, and seed as many flasks as are required, with approximately 2x10^5 cells per cm^2 when the survival rate is unknown or unpredictable, a cell count is of little value (e.g. in tumor biopsies, for which the proportion of necrotic cells may be high), in this case, set up a range of concentration from about 5-25 mg of tissue per ml.

Change the medium at regular intervals (2-4 days as detected by depression of ph.) check the supernatant for viable cells before discarding it as some cells can be slow to attach or may even prefer to proliferate in suspension.

The cold trypsin method gives a higher yield of viable cells with improved survival after 24h culture and preserves more different cell types than the warm method. Cultures form mouse embryos contain more epithelial cells when prepared by cold method, and elytroid cultures from fetal mouse liver respond to erythropoietin after this treatment but not after the warm trypsin method or mechanical desegregation. The cold trypsin method is more convenient, as no stirring or centrifugation is required, and the incubation at 4^0c may be done overnight. This method does not take longer than warm trypsin method, however, and is not as convenient when large amount of tissue are being handled. [23, 24]

iii. *Establishment of ES Cell Lines Expressing GFP under the Control of ALB Promoter / Enhancer (AG_ ES Cells):*

A 2.3-kb rat albumin (ALB) promoter/ enhancer18 was cloned into the promoterless enhanced green fluorescent protein (EGFP) vector, pEGFP-1, after digestion with SacI and KpnI restriction enzymes. The resulting construct, pALB-GFP, was electroporated into the ES cell line19 and the Hepa 1-6 ECC cell line, which was used as a positive control for GFP expression. Clones transfected with pALB-GFP were referred to as AG-ES or AG-Hepa 1-6 cells. Several independent clones were used to confirm the stable genomic integration of pALB-GFP through more than 10 passages in culture.

iv. *Culture and Differentiation of AG-ES Cells: 25, 26*

Undifferentiated AG-ES cells were maintained as described. [25] To generate embryoid bodies, the AG-ES cells were dispersed into a single-cell suspension in Iscove's modified Dulbecco's medium (IMDM; Invitrogen, Carlsbad, CA) containing 20% fetal bovine serum (FBS; HyClone, Logan, UT), 2 mmol/L L-glutamine (Invitrogen), 300 μmol/L monothioglycerol (Sigma, St. Louis, MO), and antibiotics and cultured by the hanging drop method (1×103 ES cells/30 μL). [26] After 5 days, EBs were replated on collagen IV-coated plates and cultured for an additional 26-28 days. To induce differentiation into hepatocytes, EBs were grown in the following media: IMDM supplemented with 20% FBS, 2 mmol/L Lglutamine, and 300 μmol/L monothioglycerol, and antibiotics, William E serum-free medium (Invitrogen) supplemented with 1×ITS (BD Bioscience), 10 μmol/L hydrocortisone-21-hemisuccinate (StemCell Technologies Inc., Vancouver, BC, Canada), 0.05% bovine serum albumin (Invitrogen), 2 mmol/L ascorbic acid, 10 mmol/L nicotinamide (Sigma), 1 μmol/L dexamethasone (Sigma), 2 mmol/L L-glutamine, and antibiotics; and Hepato ZYME-SFM (Invitrogen) serum-free medium designed for primary hepatocyte cultures. The media were changed every 2 days.

v. *Preparation of graft cells*

Culture dishes (9 cm in diameter), used to maintain the undifferentiated ES colonies, were washed with 8 mL of ice-cold phosphate-buffered saline (PBS, pH 7.4) 3 times and then treated with 1.0 mL of 0.025% trypsin /PBS for 2 min at 37^{0C}. Five milliliters of ES maintenance medium containing 10% FBS was added to the culture dish to stop trypsin activity. Single cell solutions were easily obtained by repeated pipetting. Cells were washed with ice-cold PBS 3 times and finally prepared for transplantation in a PBS solution at a cell concentration of 1 × 10^6cells/mL.

vi. *Infusion of embryonic stem cells*

The standard dose of Embryonic Stem cells for the treatment damaged liver of rats was calculated according to the cell viability count. Cell suspension was administered through intraperitonial route.

vii. *Grouping of experimental animals*

Wister rats weighing 175-200g were purchased from Deccan College of medical sciences, Hyderabad

and housed in polycarbonate cages and used as experimental animals. The Rats were divided into 3 groups.

Group I (n= 6) received CCl_4, 0.5 mL /kg body weight treatment and transplantation of graft cells. One day after the second injection of CCl_4 , 1 × 105 GFP-positive undifferentiated ES cells (0.1 mL of 1 × 106 cells/mL solution) were transplanted into the spleen.

Group II rats (n = 6) were injected in the same manner with 0.5 mL /kg body weight of liquid paraffin twice a week, instead of CCl_4, and transplanted with the same amount of ES cells into the spleen as Group I.

Group III rats (n= 6) were treated with CCl_4 and injected with 0.5 mL /kg body weight of liquid paraffin into the spleen in the same manner, instead of ES cells.

All the animals were sacrificed on 20th day under light ether anesthesia. The blood sample from each animal was collected separately in sterilized dry centrifuge tubes by carotid bleeding and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and subjected to biochemical investigations viz., AST, ALT, ALP, Bilirubin and total protein in serum were analyzed. Results of biochemical estimations are reported as mean±SEM of six animals in each group. The data were subjected to one-way ANOVA followed by Tukey's multiple comparision test. $P<0.001$ was considered statistically significant.

viii. *Histopathology*

The liver was excised from the animals and washed with the normal saline. The materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and processed for paraffin embedding. Sections of 5m thickness were taken using a microtome, processed in alcohol-xylene series and were stained with alum haematoxylin and eosin and subjected to histopathological examination.

III. Results

In CCl4 intoxicated rats, serum activities of AST, ALT, ALP, and Bilirubin were increased significantly when compared to the control (Table 1). The CCl_4 treated group showed a marked increase in serum bilirubin (mg %) (0.82 ± 0.08), ALT (IU/L) (222.8 ± 10.14), AST (IU/L) (254.9 ± 19.3), and ALP (IU/L) (328.5 ± 5.36) activity indicating the injury caused by CCl_4. Treatment with the Embryonic Stem cells significantly decreased the above elevated parameters and the normal architectural liver pattern was restored as given below. Liver section of control rat showed normal hepatocytes and normal architecture (Figure 1A). Liver sections from CCl_4 treated rats demonstrated Transverse section of the liver of CCl_4 treated rats showing disarrangement and degeneration of normal hepatic cells with lobular necrosis, vacuole formation and fatty change. (Figure 1B). Transverse section of the liver, after simultaneous treatment of embryonic cell lines and CCl_4 treated rats shows regeneration of hepatocytes, less vacuoles, disarrangement of fatty change compared to hepatotoxin (Figure 1C). These histopathological findings demonstrate a hepatoprotective effect of the Embryonic Stem cells against CCl_4-mediated liver damage.

IV. Discussion

The purpose of this study was to explore the hepatoprotective effect of Embryonic Stem cells in the hepatic damage caused by CCl_4. Administration of CCl_4 to normal rats increased serum levels of AST, ALT, ALP, and Bilirubin. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells.

The protective effect of the Embryonic Stem cells was further confirmed by histopathological examination of the normal control (Figure 1A), CCl_4 treated rats (Figure 1B) and Embryonic Stem cells (Figure 1C) treated. The liver of CCl_4 treated rats shows damaged liver cells. The histopathological pattern of the livers treated with CCl_4 showed a normal lobular pattern with minimal pooling of blood in the sinusoidal spaces. Positive control liver treated with Embryonic Stem cells shows mild feathery change, little bellowing degeneration of hepatocytes with normal hepatocytes. The present study reveals the hepatoprotective activity of the Embryonic Stem cells is highly efficient in hepatoprotective activity.

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. It is well documented that CCl_4 is biotransformed under the action of cytochrome P450 in the microsomal compartment of liver to trichloromethyl radical which readily reacts with molecular oxygen to form trichloromethylperoxy radical (Raucy et al, 1993). Both the radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyundaturated fatty acids (Reckngael, 1967). This leads to the formation of lipid peroxides followed by pathological changes such as depression of protein synthesis, elevated levels of serum marker enzymes such as SGOT, SGOT and ALP, depletion of glutathione content and catalase activity (Lamiyan et al, 1993) and increase in lipid per oxidation. Although serum enzyme levels are not direct measure of hepatic injury they show the status of liver. The elevated levels of serum enzymes are

indicative of cellular leakage and loss of functional integrity of cell membrane in liver.

Thus lowering of enzyme content in serum is a definite indication of hepatoprotective action of a drug. High level of SGOT indicates liver damage such due to viral hepatitis. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage. Cell lines decreased the level of both SGOT and SGPT significantly. Serum ALP levels are related to the status of and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure. In the present study it has been found to reduce serum ALP in the treated groups compared with the untreated once. Histopathological studies showed that CCl_4 caused central lobular necrosis, congestion of central vein and sinusoids. Cell lines administration exhibited protection against CCl_4 induced hepatotoxicity, which confirmed the results of biochemical studies. The results of our study indicate that administration of cell lines in CCl_4 –treated rats protects liver damage. The biochemical evaluation indicates the hepatoprotective effects of embryonic and liver cell lines shows the presence of proliferating cells which may be responsible for proper physiology of liver.

V. Conclusion

To conclude, the Embryonic Stem cells showed significant hepatoprotective activity which is confirmed by estimating the liver enzymes. Further the results are supported by histopathological studies indicating the reparative effect of Embryonic Stem cells in comparison with positive control. Any how the work under taken is a stepping stone for our future studies leading to stem cell isolation and subjecting them for the treatment of various ailments.

Table 1 : Assessment of serum biochemical parameters in CCl_4 induced hepatic injury and regenerative effect of undifferentiated embryonic stem (ES) cells in rats.

Group	Total bilirubin (mg/dl)	Total protein (gm %)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Group – I CCl_4+0.1 mL of 1 × 10^6 cells/mL solution	0.33± 0.15 [b]	7.46± 0.02	105± 13.14 [b]	90± 11.15 [b]	238 ± 10.20 [b]
Group – II 0.5 mL /kg of liquid paraffin	0.11 ± 0.02	9.44 ±0.02	88.17 ± 5.47	54 ± 2.7	249.5 ± 18.2
Group –III CCL_4+0.5 mL /kg of liquid paraffin	0.82 ± 0.08 [b]	6.93 ±0.01	254.9 ± 19.3[a]	222.8 ± 10.14[b]	328.5 ± 5.36 [b]

Values are expressed as mean±SEM. aP < 0.05, bP < 0.01 vs control.

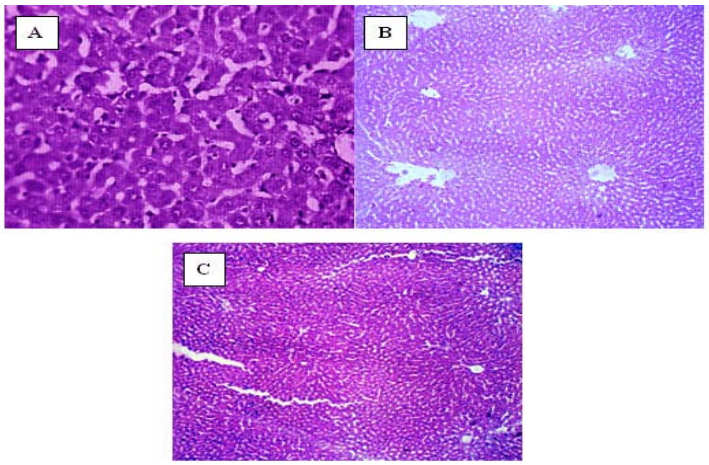

Figure 1 : a) Transverse section of the liver of normal control rats, showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein. b) Transverse section of the liver of CCl_4 (0.5 ml/kg) treated animals showing disarrangement and degeneration of normal hepatic cells with lobular necrosis, vacuole formation and fatty change. CV: Central vein; HC: hepatocytes; SS: Sinusoidal space; c)Transverse section of the liver, after simultaneous treatment of Embryonic Stem cells and CCl_4 treated animal' s shows regeneration of hepatocytes, less vacuoles, disarrangement of fatty change compared to hepatotoxin.

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Design and Evaluation of a 3-Component Composite Excipient "Microcrystarcellac" as a Filler-Binder for Direct Compression Tabletting and it's Utilisation in the Formulation of Paracetamol and Ascorbic Acid Tablets

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Abstract - A research was conducted to design and evaluate a highly functional 3-component composite fillerbinder for direct compression. Tapioca starch (NTS) was modified physically at molecular level by annealing and enzyme hydrolyzed to obtain microcrystalline tapioca starch (MCTS) which was coprocessed with LMH and microcrystalline cellulose (MCC) to yield Microcrystarcellac (MSCL). NTS was extracted from cassava tuber (Mannihot esculenta crantz) using a standard method. The powder suspensions were prepared in concentration of 40 %w/w in five separate conical flasks. The starch granules were annealed for 1 h and subsequently hydrolyzed with α-amylase at 58o and pH 7 for 1, 2, 3, 4, and 5 h in a water bath. The reaction was terminated and neutralized with 0.1 N HCL and 0.1 N NaOH respectively. The MCTS was washed, recovered by sedimentation and air dried at room temperature for 72 h.

Keywords : Microcrystarcellac, Coprocessed Excipient, Directly compressible Excipient, Highly functional Filler-binder, Microcrystalline Tapioca Starch.

GJMR-B Classification : NLMC Code: 110405

Strictly as per the compliance and regulations of:

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Shittu, A.O.[α] , Oyi, A.R.[σ], Isah, A.B.[ρ] & Ibrahim, M.A[ω]

Abstract - A research was conducted to design and evaluate a highly functional 3-component composite filler- binder for direct compression.

Tapioca starch (NTS) was modified physically at molecular level by annealing and enzyme hydrolyzed to obtain microcrystalline tapioca starch (MCTS) which was coprocessed with LMH and microcrystalline cellulose (MCC) to yield Microcrystarcellac (MSCL). NTS was extracted from cassava tuber (*Mannihot esculenta crantz*) using a standard method. The powder suspensions were prepared in concentration of 40 %w/w in five separate conical flasks. The starch granules were annealed for 1 h and subsequently hydrolyzed with α-amylase at 58o and pH 7 for 1, 2, 3, 4, and 5 h in a water bath. The reaction was terminated and neutralized with 0.1 N HCL and 0.1 N NaOH respectively. The MCTS was washed, recovered by sedimentation and air dried at room temperature for 72 h. Following characterization, the granules that were modified for 3 h, sieved fraction >75-250 μm was coprocessed with α- lactose monohydrate(α-LMH) and Microcrystalline cellulose (MCC) at concentrations of 10-50 % (MCTS), 45-25 %(α-LMH) , 45-25 %(MCC). Granule size ranges >75 - 250 μm, and >90 - 250 μm were characterized and compacted at a range of compression load 2.5 to 12.5 KN.

Average flow rate, angle of repose and carr's index were 2 g/s, 31.6º, 13.4 % respectively for MSCL (granule size range >90 - 250 μm and component ratio of MCTS, α-LMH, and MCC is 20: 40:40). The corresponding values for the direct physical mixture of MCTS, α-LMH and MCC are 0.45 g/s, 47.5º, 52 % respectively. MSCL have improved functionality over direct physical mixture of the primary excipients. MSCL was compared with Starlac®, and Cellactose®. The onset of plastic deformation Py (yield value) are: MSCL (22.3 MNm-2)>Cellactose (24.2 MNm-2)> Starlac (143 MNm-2). The degree of plastic deformation occurring during compression (Pk) is in the following order: MSCL (16.3 MNm-2)> Cellactose® (17 MNm-2)>MCC (18.6 MNm-2)> Starlac® (19.1 MNm-2). MSCL is more superior in functionality than Starlac, and Cellactose. The dilution potential obtained for MSCL compacted with paracetamol (PCM) and ascorbic acid (AA) as active drug (API) are: 50 % AA with MSCL, 45 % PCM with MSCL. The hardness of MSCL containing 45 % PCM, 70 N; MSCL containing 50 % AA, 68 N. MSCL can be used to formulate tablets of both poorly compressible API and moisture sensitive API. Kitazawa dissolution rate constant, KD at t = 10min. follow this order:MSCL – AA (11.0 x 10-3 mg min-1) > Cellactose – AA (10.3 X 10-3).Cellactose – PCM (9.3 x 10-3 mg min-1) > MSCL – PCM (7.5 x 10-3mg min-1).

Keywords : Microcrystarcellac, Coprocessed Excipient, Directly compressible Excipient, Highly functional Filler-binder, Microcrystalline Tapioca Starch.

I. Introduction

The *growing performance expectations of excipients* to address issues such as flowability, compactibility, disintegration, dissolution and bioavailability also placed a demand for newer excipients with high functional property.

Co-processing excipients lead to the formation of excipient granulates with superior properties compared with physical mixtures of components or with individual components. They have been developed primarily to address the issues of flowability compressibility, and disintegration potential, with filler-binder combinations being the most commonly tried. The combination of excipients chosen should complement each other to mask the undesirable properties of individual excipients and, at the same time, retain or improve the desired properties of excipient. For example, if a substance used as a filler-binder has a low disintegration property, it can be coprocessed with another excipient that has good wetting properties and high porosity because these attributes will increase the water intake, which will aid and increase the disintegration of the tablets.

Material science plays a significant role in altering the physicomechanical characteristics of a material, especially with regard to its compression and flow behaviour. Coprocessing excipient s offers an

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interesting tool to alter these physicomechanical properties. Materials, by virtue of their response to applied forces, can be classified as elastic, plastic, or brittle materials.

Pharmaceutical materials exhibit all three types of behavior, with one type being the predominant response. Coprocessing is generally conducted with one excipient that is plastic and another that is brittle.. This particular combination prevents the storage of too much elastic energy during compression, which results in a small amount of stress relaxation and reduced tendency of capping and lamination[1]. A combination of plastic and brittle materials is necessary for optimum tabletting performance. Hence, coprocessing these two kinds of materials produces a synergistic effect, in terms of compressibility, by selectively overcoming, the disadvantages. Such combinations can help improve functionalities such as compaction performance, flow properties, strain-rate sensitivity, lubricant sensitivity or sensitivity to moisture or reduced hornification.

II. Materials and Methods

a) Materials

Cassava tuber (*Mannihot esculenta crantz*) obtained from University of Agriculture Abeokuta, Ogun State, Nigeria. Phloroglucinol, iodine, xylene, Starlac (Roquette, France), Cellactose
(Meggle, Germany), Microcrystalline cellulose (Avicel 101).

b) Methods

i. *Extraction of Tapioca Starch2*

Cassava tubers were washed and peeled to remove the outer skin and rind with the aid of a handy stainless knife. The peeled tubers were washed with freshly distilled water and rasped.

The rasp consists of a sheet of metal plate perforated with nails, clamped around a stainless bucket with the protrusions facing outwards. The tubers were then manually rasped to a pulp on the stationary grater (which is the metal plate perforated by nails). Water was applied in small quantities continuously to the rasper. The process was continued until the whole tubers were turned into a fine pulp in which most but not all of the starch granules were released.

After rasping, pulp from the sump was then pumped on to a nylon fastened /clamped around a stainless bucket. A small spray of water was applied to assist the separation of starch granules from their fibrous matrix and to keep the screen mesh clean while water was added, the mass were turned manually to aid the release of the granules. Starch granules carried with the water fall to the bottom of the bucket in which the sieve was placed. The starch milk was then allowed to sediment, by standing for a period of 8 h. The starch settled at the bottom of the bucket and the supernatant liquor decanted. The sediment / fine granules were centrifuged. After the removal of free water from the starch, cake was obtained. The starch cake was then crumbled into small lumps (1-3 cm) and spread out in thin layers on stainless trays and air dried for 120 h [2,3].

ii. *Preparation of microcrystalline Tapioca Starch (MCTS)* [4]

Five hundred gram (500 g) of tapioca starch granules were weighed into five places and each placed in a 1000 ml capacity conical flask. Six hundred millimeters (600 ml) of freshly distilled water was added to each content of the flask to make a suspension (= 40 %w/w). The pH of the medium was adjusted to between 6.5 and 7.0. All the flasks were placed on a digitalized water bath and the starches were annealed at 60°C for 30 min. Each flask was dosed with 0.5 ml of α-amylase (0.1 % v/w d.s) at 60oC on water bath and was allowed to stand for hydrolysis to take place at various length of specified time: 60, 120, 180, 240, and 300 min). At the end of the first 60 min., the enzyme reaction in one of the flasks was terminated by adjusting the pH to 2.0 with 0.4 N HCL after which the pH was raised to 6.5 with 0.4 N NaOH. The medium was filtered through a Buckner funnel; the residue was washed 3 times, with distilled water and finally dehydrated by adding enough isopropanol (99 %) (a water – miscible solvent) and the resulting dehydrated highly crystalline starches were air dried . These procedures were repeated for the remaining hydrolyzed starches at other times.

iii. *Preparation of Three Component Composite Filler- Binder (Microcrystarcellac) by Codried method.*

Table 1 : The working formula for preparation of the novel three component composite excipient (microcrystarcellac).

Material	Batch				
	% (w/w)				
	1	2	3	4	5
MCTS (g)	45	40	35	30	25
Lactose (g) (α – L-MH)	45	40	35	30	25
MCC (g)	10	20	30	40	50

The slurry form of annealed enzyme hydrolyzed tapioca starch (MCTS) (sieved fraction, <75 μ) was coprocessed with α- lactose monohydrate (α – L-MH) (sieved fraction, <75 μ), and microcrystalline cellulose (MCC) (sieve fraction, <75 μ). The slurry was made by suspending the MCTS in a solution of Isopropranol and freshly distilled water in ratio 2:1 respectively. MCTS slurry was blended with α – L-MH, and MCC at concentrations indicated in Table 1 as a dried mass relative to MCTS. The composite slurry was stirred vigorously with a stirrer until a semi-solid mass easily ball was formed. The composite mass was then granulated through a 1500 μ and codried at 60oC until a constant weight was reached. Codried granules were pulverized and sized by passing through mesh size 500 μm, and the fraction between >75 – 250 μm was reserved. The powder and tabletting properties of the codried products were evaluated and compared to those of corresponding components and physical mixtures.

iv. *Compactibility*

The preliminary study was carried out to select few promising batches: (1) the best batch out of the five batches of hydrolyzed starch (MCTS) having the best tablet properties to be coprocessed with lactose and MCC, (2) the best two batches (out of five) of coprocessed filler-binder for microstructuring before compaction studies.

The native tapioca starch, and the microcrystalline tapioca starch at various time of hydrolysis were compressed on a single punch Erweka tabletting machine (Erweka, AR 400. Germany), fitted with 10.5 mm diameter flat faced punch and die. Tablet target was 500 mg, and pressure load used range from 4 to 7 KN.

The coprocessed filler-binder: MSCL (5 batches each) were subjected to the same procedure to streamline the batches to just two for effective research and particle restructuring. The batches chosen here were subjected to particle sieving and further employed for compaction studies.

v. *Compaction Studies*

a. Preparation of Compacts

Compacts of weights, 500 mg, of each of the primary powders [tapioca starch, microcrystalline cellulose (MCC), lactose], annealed tapioca starch (ATS), annealed enzymatically hydrolyzed tapioca starch (MCTS), Microcrystarlac (B4 and B5), Microcrystarcellac (B2 and B3), physical mixture of MCTS and lactose; MCTS, lactose and MCC, were made using a single punch carver hydraulic hand press (model, C, Carver Inc. Menomonee Falls, Wisconsin, U.S.A) at machine compression force ranging from 2.5 KN to 12.5 KN. Fourty compacts were made at each compression level for individual material. Before compression, the die (10.5 mm diameter) and the flat faced punches were lubricated with a 1 % w/v dispersion of magnesium stearate in ethanol-ether (1:1). The compacts were stored over silica gel for 24 hours (to allow for elastic recovery and hardening and to prevent falsely low yield values) before evaluations. The dimensions (thickness and diameter) and weight uniformity of ten compacts were determined. The relative density, D, were calculated as the ratio of density of the compact, Dt to the particle density, Dp of individual powder or composite. The data obtained using 'ejected tablet method (out-of-die)' were used to obtain the Heckel plots.

The weights, W, and dimensions were then determined respectively, and their relative densities, D, were calculated using the equation:

$$D = W/ [V_t \times P_s] \quad \text{----------(1)}$$

Where V_t is the volume of the tablet in cm^3, and P_s is the particle density of the solid material in gcm^{-3}.

Heckle plots of ln (1/ 1 – D) versus applied pressure "P"[6] and Kawakita plots of P/C versus P,[7] were constructed for the composite excipients.

Linear regression analysis was carried out over a compression range 2.5, 5, 7.5, 10, and 12.5 KN. The parameters from Heckel plots were calculated. The Kawakita equation was employed to determine the extent of plastic deformation the material undergoes.

b. Moisture content

The moisture content (MC) of the powder was determined by weighing 100 g of the powder after which it was heated in an oven at a temperature of 105 °C until a constant weight was obtained.

The moisture content was then calculated with the following formula:

$$MC = (1 - W_t/W_0) \times 100 \quad \text{----------(2)}$$

Where W_t and W_0 represent weight of powder after time ' t' and the initial weight before heating respectively.

vi. *Determination of Flow Rate and Angle of Repose*

Angle of repose was determined using a standard method and equation 3 bellow.

$$\theta = \tan^{-1} (h/r) \quad \text{.....(3)}$$

The flow rates were determined with the aid of Erweka flowability tester (model GDT, Germany).

vii. *Densities*

a. True (particle) densities

The true (particle) densities of the primary powders (tapioca starch and mcc-derived), annealed starch, annealed enzymatically hydrolyzed tapioca starch and the composite particles were determined by the liquid displacement method using a specific gravity bottle with Xylene as displacement fluids, and the particle density, D_p, computed according to the following equation:

$$D_p = W/[(a + W) - b] \times SG \quad \text{----------(4)}$$

Where, W, is the weight of powder, SG, is the specific gravity of the solvent, a, is the weight of bottle plus solvent, and, b, is the weight of bottle plus solvent plus powder. The measurement was performed in triplicate.

b. Bulk and Tap density

Bulk density[8]

These parameters were determined by weighing 50 g quantity of each granule/powder and pouring into a 100 ml measuring cylinder. The volume (V_o) was recorded as the bulk volume. The total weight of the granule/powder was noted. The bottom of the cylinder was raised 10 cm above the slab and made to fall on the platform continuously for 100 taps. The volume of (V_t) of the granule was recorded, and this represents the volume of the granules minus the voids and is called the tapped volume. The final weight of the powder too was recorded as the tapped weight.

The bulk and tapped densities were calculated as:

$$B_d = W/V_o \quad \text{----------(5)}$$

$$B_t = W/V_t \quad \text{----------(6)}$$

Where, B_d and B_t, are bulk and tapped density respectively, and W, is the weight of the powder (50 g).

The results presented are the mean of three determinations.

Carr's Index

$$\text{Carr's Index (CI)} = (\rho^T - \rho^o)/\rho^o \times 100\ \% \quad \text{----------(7)}$$

Where ρ^o is the poured or bulk density and ρk is the tapped density.

viii. *Evaluation of Tablets*

Weight variation Limit Test: The weights of 10 tablets were determined individually and collectively on a Metler balance (Denver, XP-300, U.S.A). The mean weight, percentage (%) deviation from the mean and standard deviation were calculated.

a. Thickness of Tablets

The thickness of the tablets was measured with the aid of micrometer screw gauge. Five tablets were selected randomly and the thickness for each was measured and the mean value determined.

b. Hardness of tablets

Crushing strength was determined using an electronic/digitalized tablet hardness tester (model EH O1, capcity 500 N,Indian).

c. Friability

The friability test was performed for the tablets formulated in a friabilator (Erweka, TA 3R). The weight of 10 tablets was determined on a Metler balance (Denver, XP - 300, U.S. A). The tablets were placed in the friability and set to rotate at 25 r.p.m for 5 min after which the tablets were de-dusted gently and their weight determined. The difference was calculated and the percentage loss in weight and hence the value of the friability was calculated.

Compact Volume: The volume of a cylindrical tablet having radius 'r' and height 'h' is given by the following equation.

Compact density: The compact density of a tablet was calculated from the following equation.

$$Vc = h\pi r^2 \quad \text{..... (8)}$$

$$\text{Compact density } (\rho) = \frac{\text{Weight of tablet}}{\text{Volume of tablet}} \quad \text{..... (9)}$$

d. Compact Radial tensile strength[9]

The tensile strength of the normal tablets (T) was determined at room temperature by diametral compression[9] using an hardness tester (model EH O1, capacity 500 N, Indian) and by applying the equation :

$$T = 2\,F / (\pi dt) \quad \text{----------(10)}$$

Where T is the tensile strength of the tablet (MNm-2), F is the load (MN) needed to cause fracture, d is the tablet diameter (m). Results were taken from tablets which split cleanly into two halves without any lamination. All measurements were made in triplicate, and the results given are the means of several determinations.

Compression pressure: This was derived from the relationship between the applied pressure and surface area.

$$\text{C.P.} = \frac{\text{Applied force}}{\text{Surface area of tablet}} \quad \text{.....(11)}$$

e. Disintegration Time

Disntegration apparatus (Erweka, ZT 3, Germany) was employed. Three tablets were placed in each compartment of the disintegration basket which was lowered into a glass beaker (1 L capacity) filled with deionized water to 800 ml mark and in turn was placed in a water bath maintained at 37°C. The time taken for the disassociated tablet particles to pass through the mesh was recorded as the disintegration time. Average of three readings was taken as the disintegration time.

ix. *Determination of dilution capacity*

Ascorbic acid and paracetamol were used as model drugs representing both highly water soluble, moisture sensitive, and elastic/poorly water soluble active ingredient respectively.

Model drugs were blended in deferent ratios, ranging from 0 %, 5 %, 10 %, up to 50 % with MCTS, microcrystarlac and microcrystarcellac.

Formulations were blended by method of dilution and lubricated with 1 % magnessium stearate. Each batch was compressed for 30 seconds on single punch Carver hydrolic hand press(model, C, Carver Inc. Menomonee Falls, Wisconsin, U.S.A) at pressure load of

7.5 KN, target weight of 500 mg. Compacts were allowed to relax for 24 h post compression. Compact dimensions (diameter and thickness) were determined using a digitalized vernial caliper. Crushing strength was determined using an electronic/digitalized tablet hardness tester (model EH O1, capcity 500 N,Indian). A relationship between amount in percent (%) of model drug added to the formulation and the tensile strength will be generated.

In general, the capacity was expressed by the dilution potential as being an indication of the maximum amount of active pharmaceutical ingredient that can be compressed with the excipient, while still obtaining tablets of acceptable quality (that is, acceptable crushing strength average of 60 N, friability, < 1.0 %, good disintegration time < 15 min, and must meet the requirement of U.S.P weight variation limit test).

III. Results and Discussion

Table 2 : Shows Powder characteristics of primary excipients, coprocessed filler-binder and standard coprocessed filler-binder

Material	Flow rate g/sec (o)	Angle of Repose	Bulk Density g/cm³	Tapped Density g/cm³	Compressibilty index %	Hausner Ratio
NTS	2	43.4	0.545	0.817	50	1.5
MCTS(>75-250 μm)	2.5	24.5	0.516	0.712	38	1.4
MSCL-B2 (>90-250μm)	2	31.6	0.677	0.768	13.4	1.13
MSCL-B2 (>75-250μm)	1.8	37	0.555	0.758	36.6	1.4
MSCL-B3 (>90-250μm)	1.8	31	0.483	0.744	54	1.5
MSCL-B3 (>75-250μm)	1.6	32	0.526	0.685	30	1.3
MCTS+LMH+MCC B_2 (40:40:20) (Physical mixture)	0.45	47.8	0.481	0.735	52	1.53
Starlac®	7.1	19.2	0.641	0.725	13.1	1.13
cellactose®	1.84	24.2	0.443	0.532	20.1	1.2

NB. MSCL, MCTS, NTS, LMH, and MCC represent: microcrystarcellac, microcrystalline tapioca starch, native tapioca starch, α-lactose monohydrate, and microcrystalline cellulose. B2 and B3 represent batch 2 and batch 3. Batch 2 consist of MCTS, LMH, and MCC in ratio 40 %, 40 % and 20 % respectively; while batch 3 consist of MCTS, LMH, and MCC in ratio 35 %, 35 % and 30 % respectively.

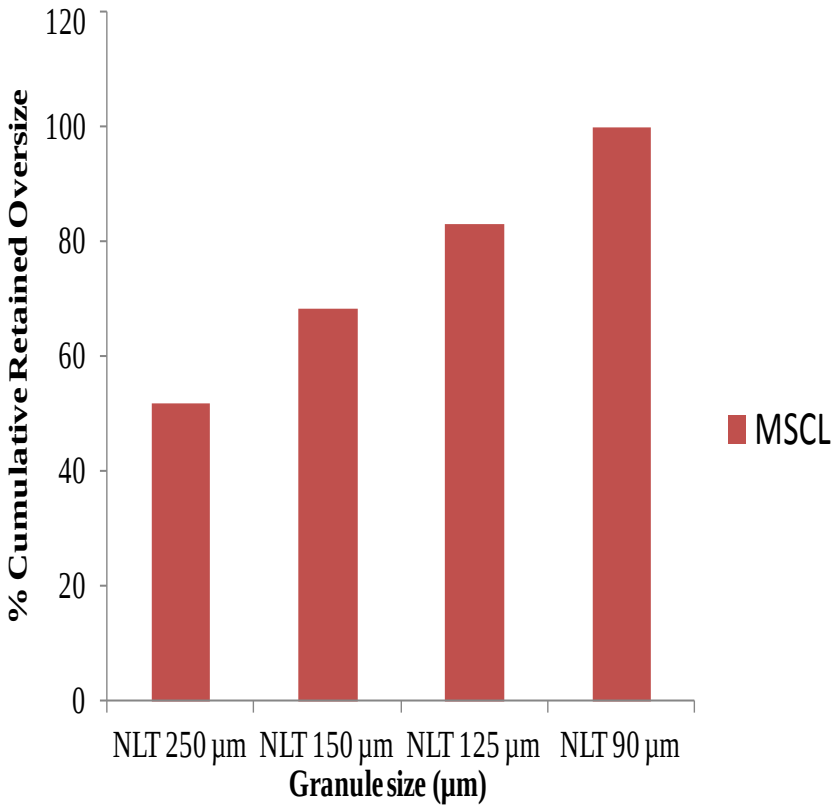

Figure 1 : Shows MSCL (40:40:20) granule distribution in percent cumulative retained oversize versus granule size(NLT: Not Less.

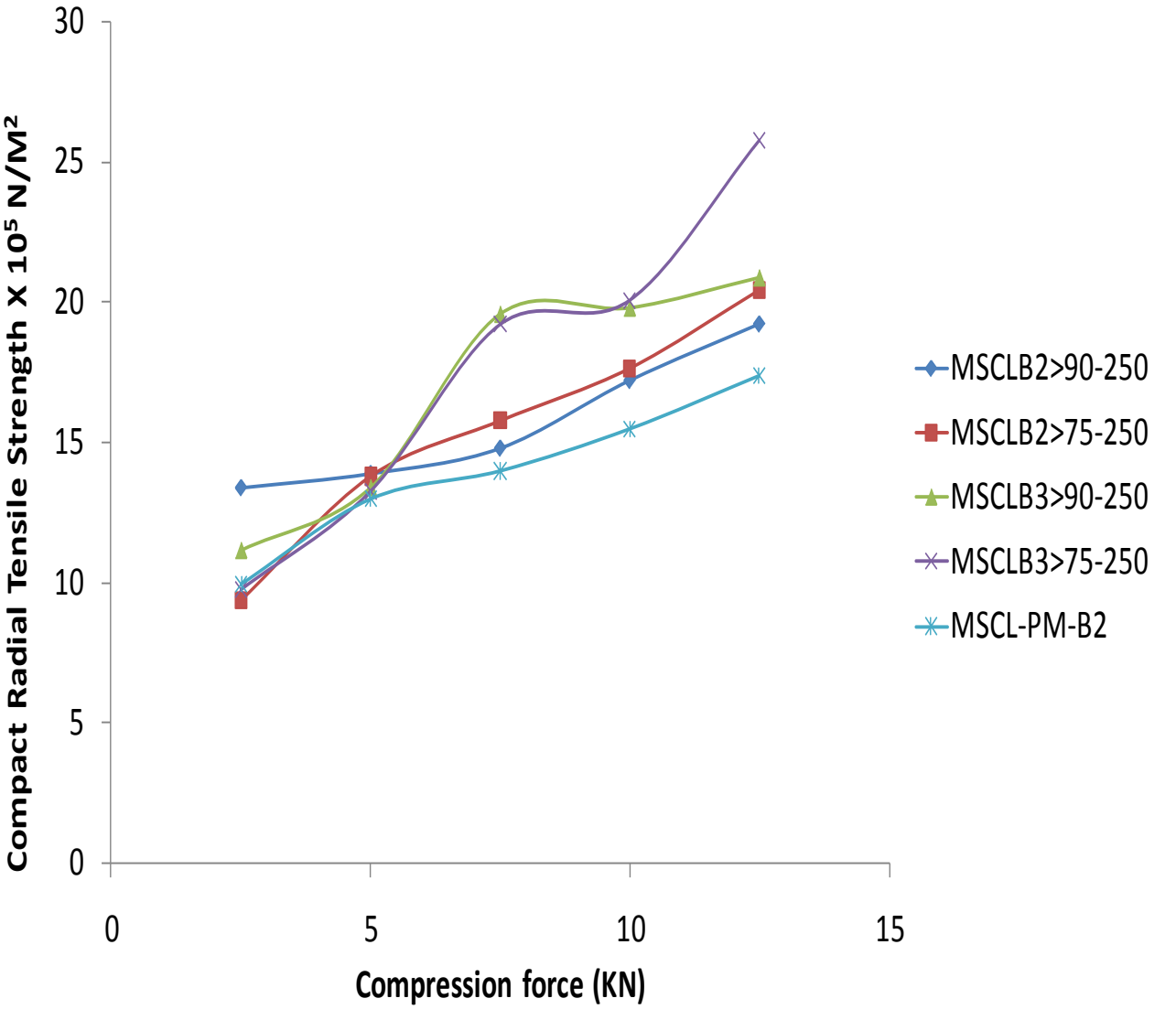

Figure 2 : Shows the effect of compression pressure on tensile strength of coprocessed microcrystarcellac (MSCL), and direct physical mixture (MSCL-PM-40:40:20) placebo tablets.

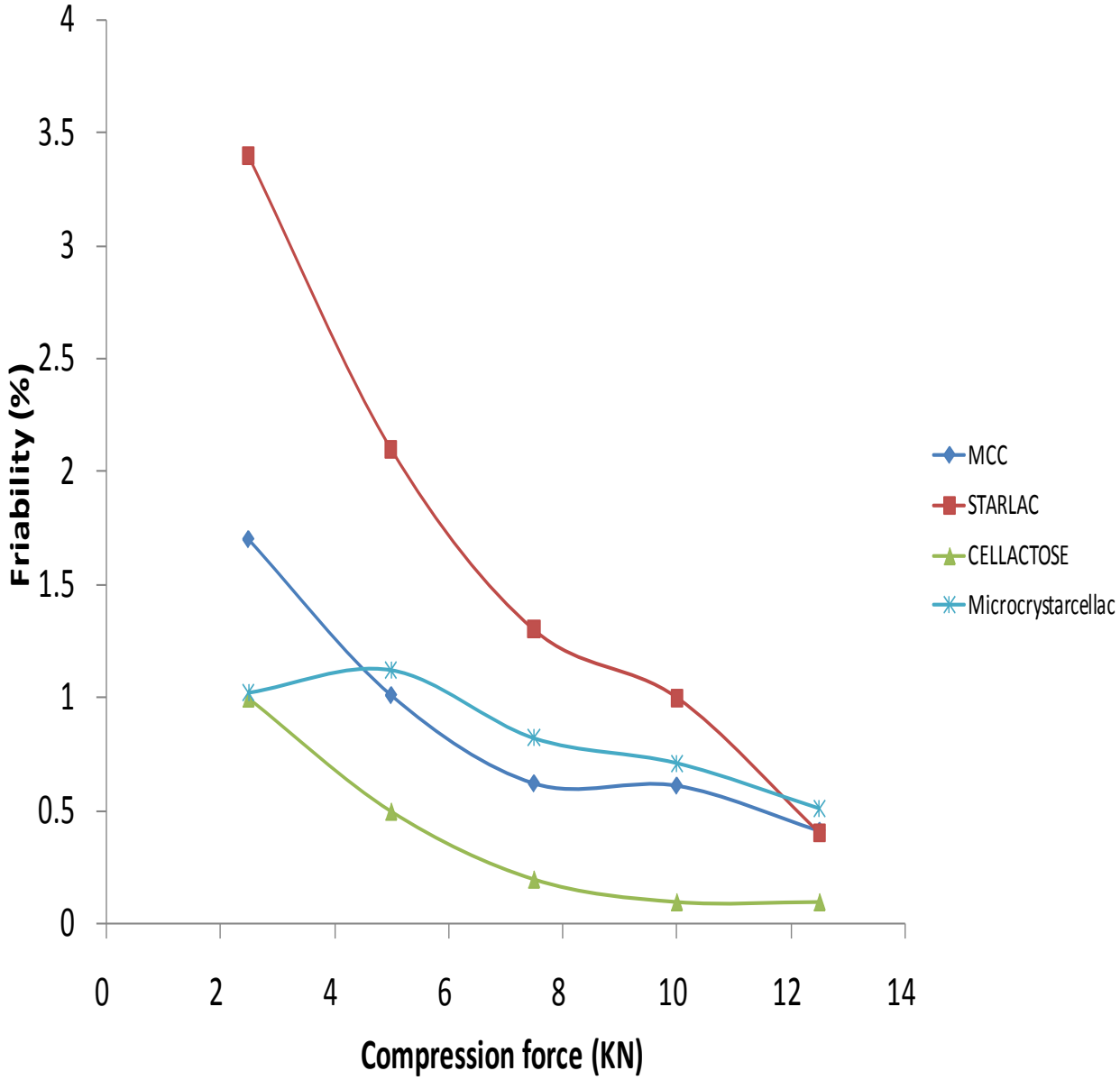

Figure 3 : Shows the effect of increasing compression force on friability of, MSCL, Starlac and Cellactose (Placebo tablets).

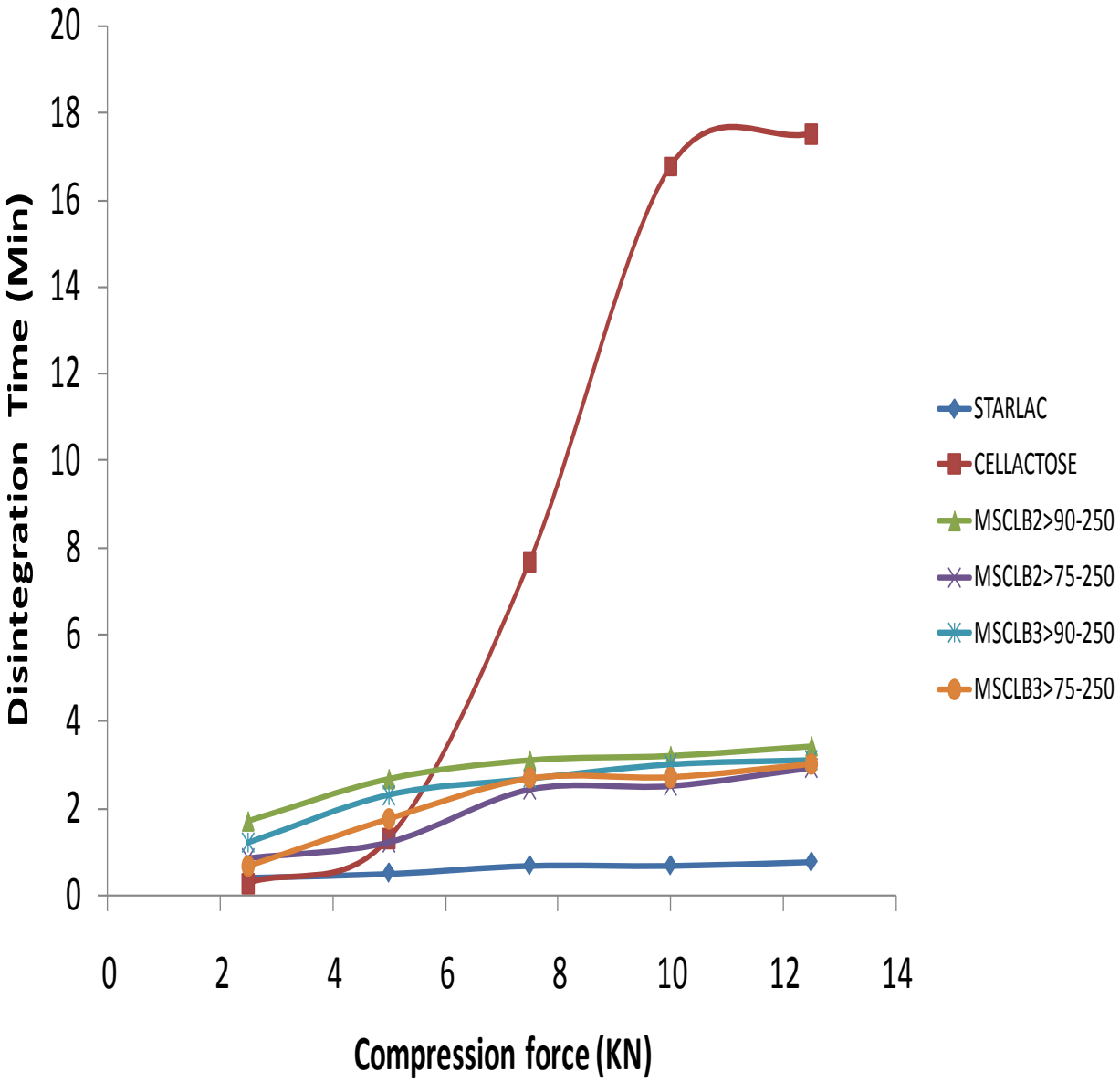

Figure 4 : Shows the effect of increasing compression pressure on disintegration time of Placebo Compacts containing microcrystarcellac (MSL), Starlac, and Cellactose.

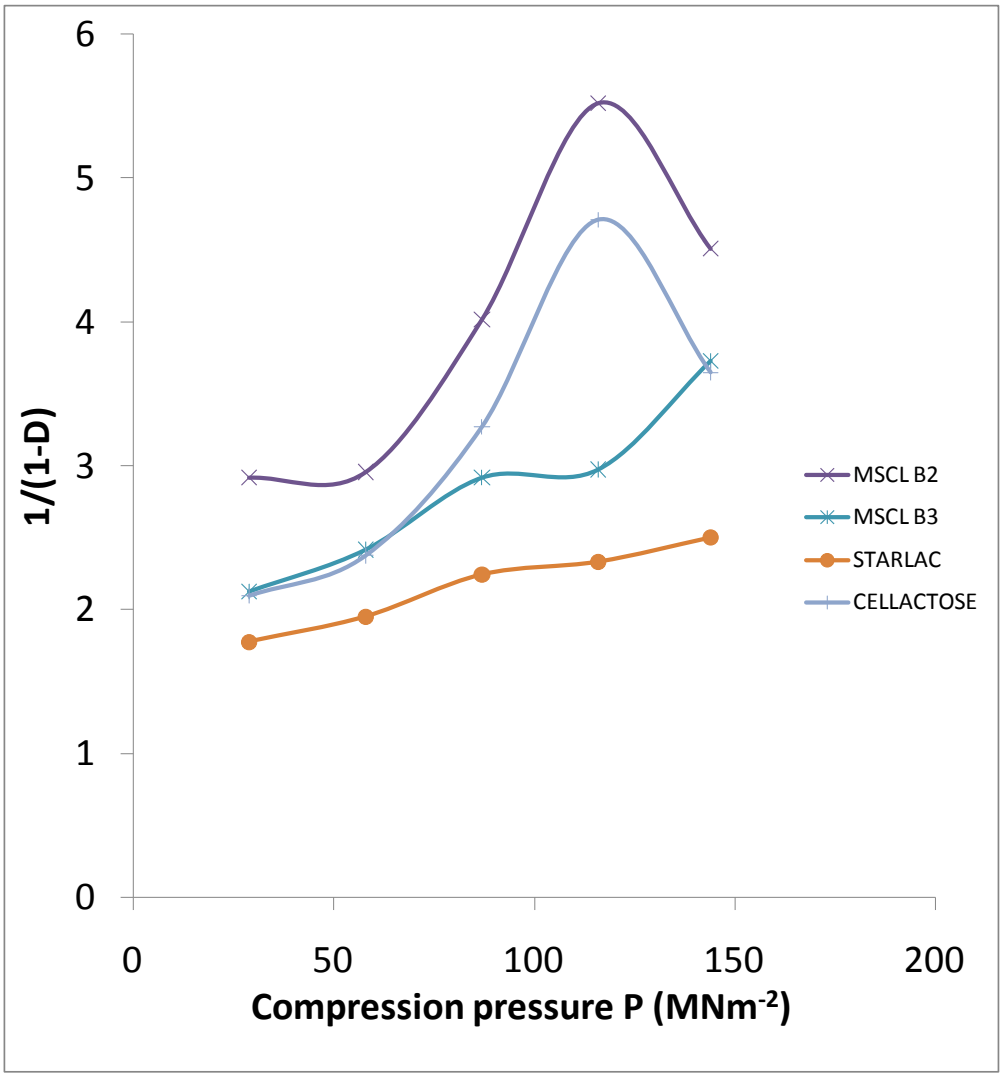

Figure 5 : Shows the effect of increasing compression pressure (P) on volume reduction \[1/(1-D)]ofplacebocompact ofmicrocrystarcellac (MSCL), Starlac, Cellactose, microcrystalline cellulose (MCC).

Table 3 : Shows the parameter obtained from Heckel Plots for Composite Particles, MSCL, Starlac®, Cellactose® and MCC.

Material	K	P_Y (MNm^{-2})	A	e^{-A}	D_o	D_A	D_B
Microcrystarcellac (B2)	0.048	22.3	1.0	0.368	0.470	0.632	0.162
Starlac	0.007	143	1.7	0.183	0.413	0.817	0.404
Cellactose	0.041	24.2	0.6	0.545	0.298	0.455	0.157

NB: A and K represent: constants of Heckel equation. PY represent: mean yield value. Do, DA, and DB represent: initial rearrangement phase of densification, total degree of densification at zero pressure and rearrangement phase of particles in the early stages of compression respectively.

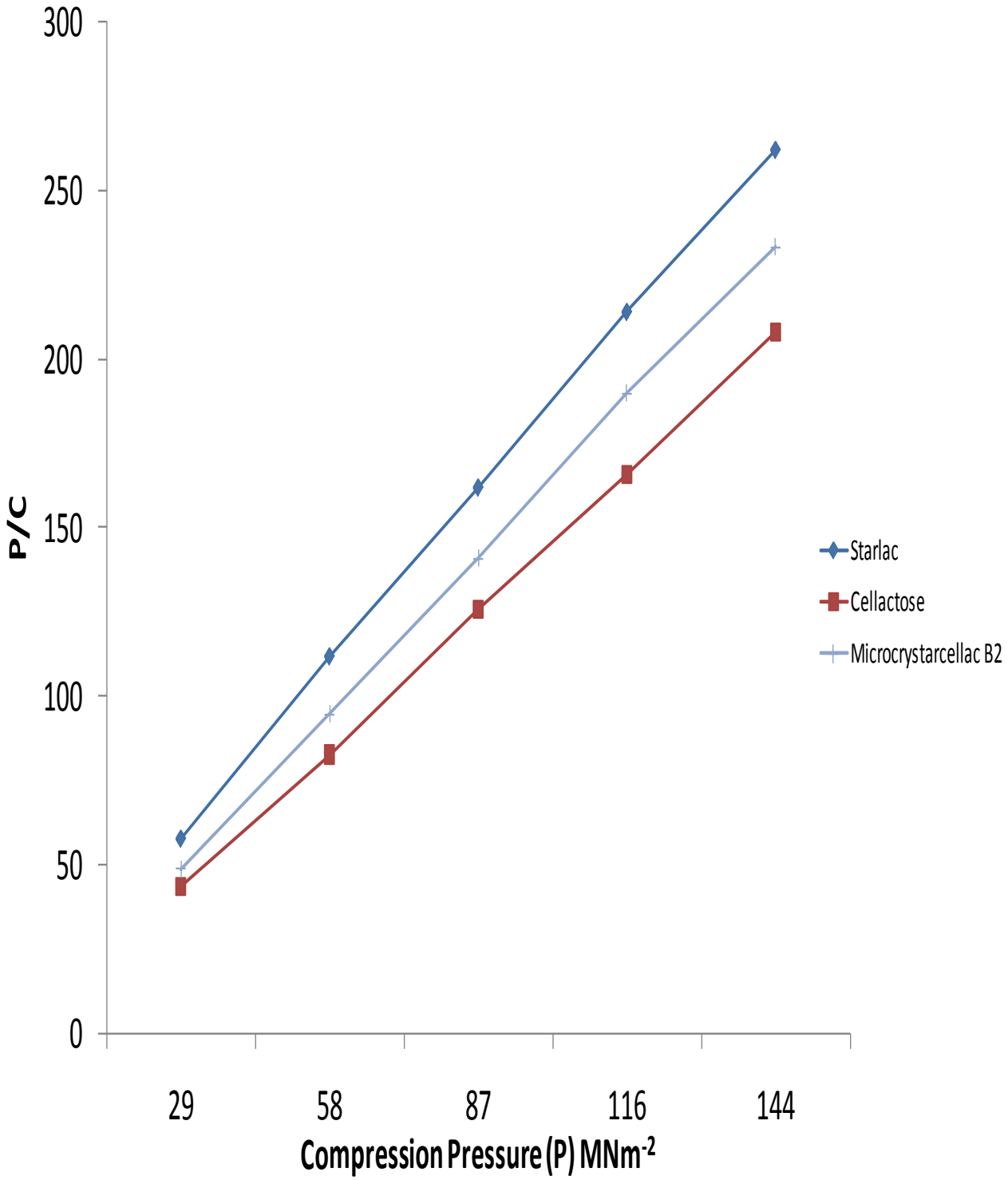

Figure 6 : Kawakita analysis of placebo compact of, Microcrstarcellac(MSCL), Starlac, Cellactose and Microcrystalline cellulose (MCC).

Table 4 : Shows the parameters obtained from Kawakita plot analysis.

Material	a	1/a	$D_i = (1 - a)$	1/b	$P_K(MNm^{-2})$
Cellactose®	0.526	1.9	0.474	17	17
Starlac®	0.714	1.4	0.286	19.1	19.1
Microcrystarcellac B_2 (40:40:20)	0.610	1.64	0.390	16.3	16.3

NB: 'a' and 'b' are constants of Kawakita equation ('a' gives minimum porosity of the bed prior to compression, while 'b' gives the coefficient of compression is related to the plasticity of the material). Di indicates the packed initial relative density of tablets formed with low pressure. Pk gives and inverse measurement of plastic deformation occurring during compression.

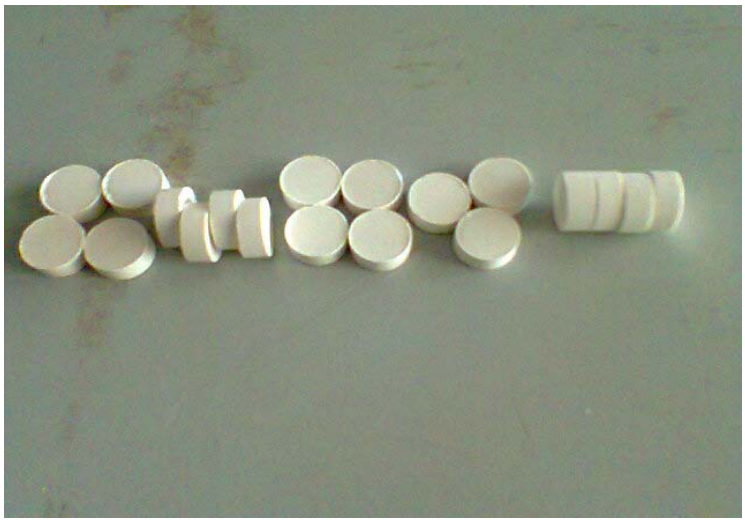

Figure 7 : Shows photograph of placebo tablets of Microcrystarcellac,(MSCL-B2).

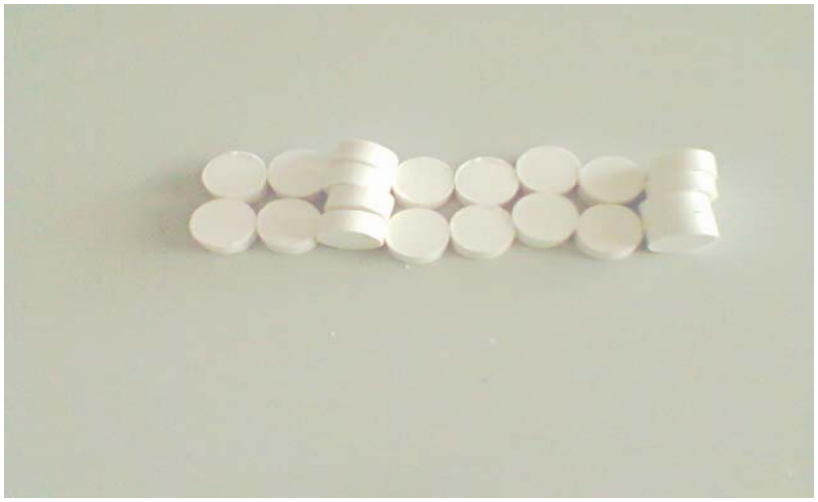

Figure 8 : Shows photograph of tablets containing Microcrystarcellac 55 % and Paracetamol 45 %, (MSCL-PCM 45%).

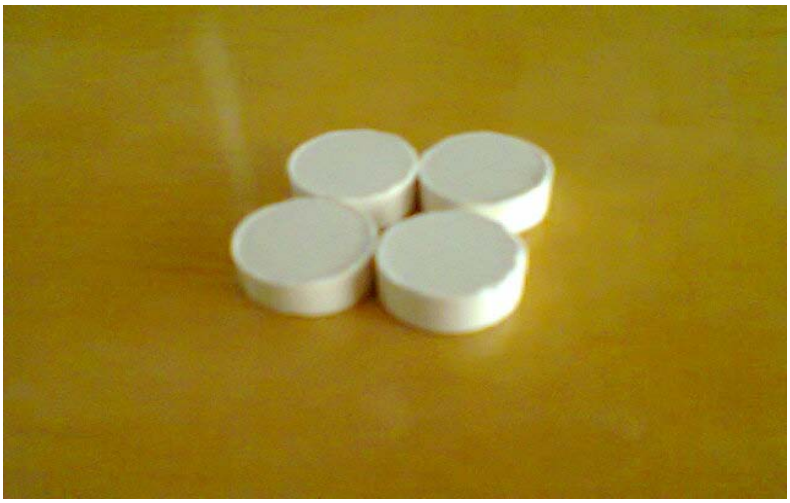

Figure 9 : Shows photograph of tablets containing Microcrystarcellac 50 % and Ascorbic Acid 50 %, (MSCL-AA-50%).

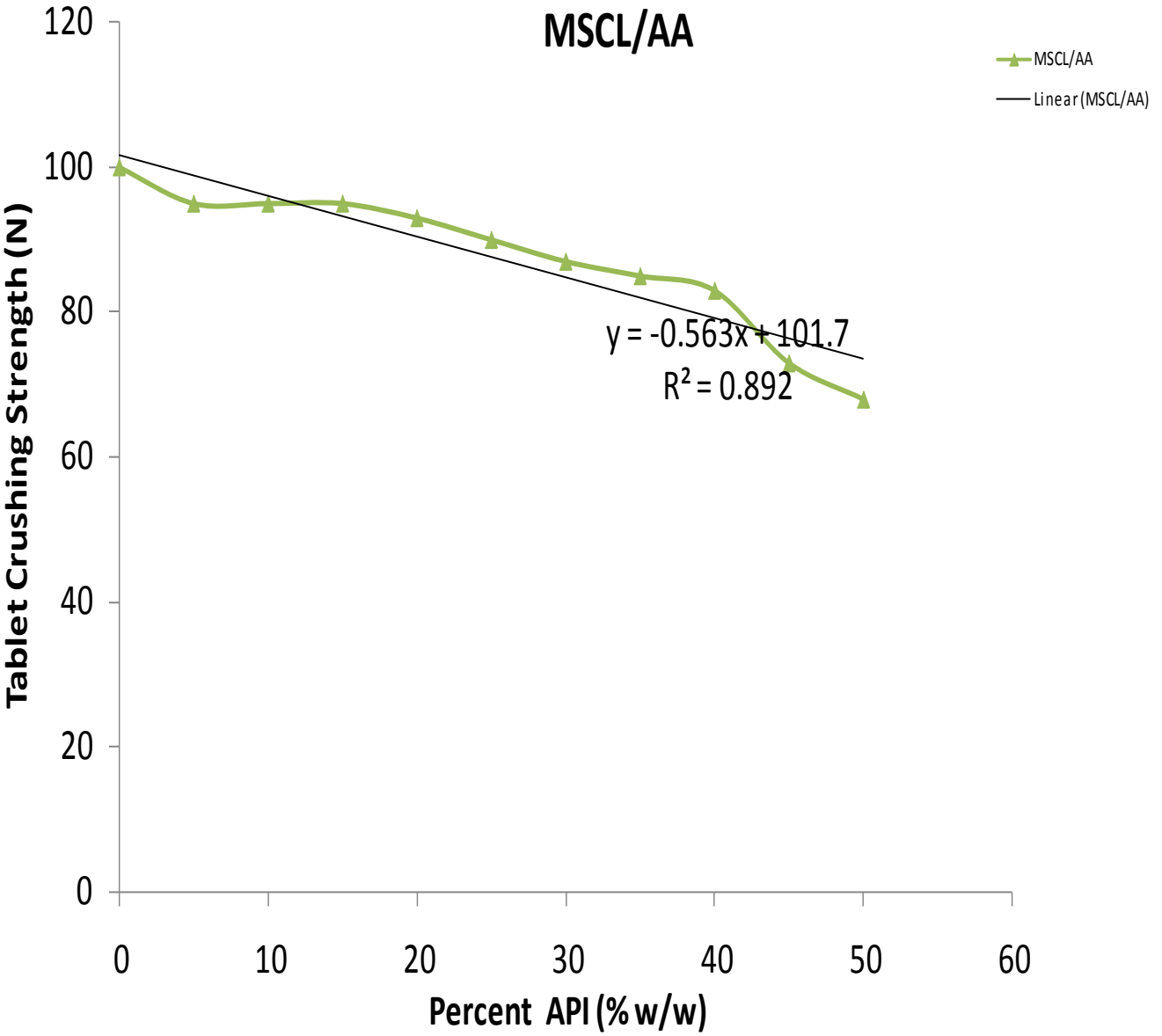

Figure 10 : Shows the effect of increasing percentage of ascorbic acid(AA)on tensile strength of MSCLcompacts.

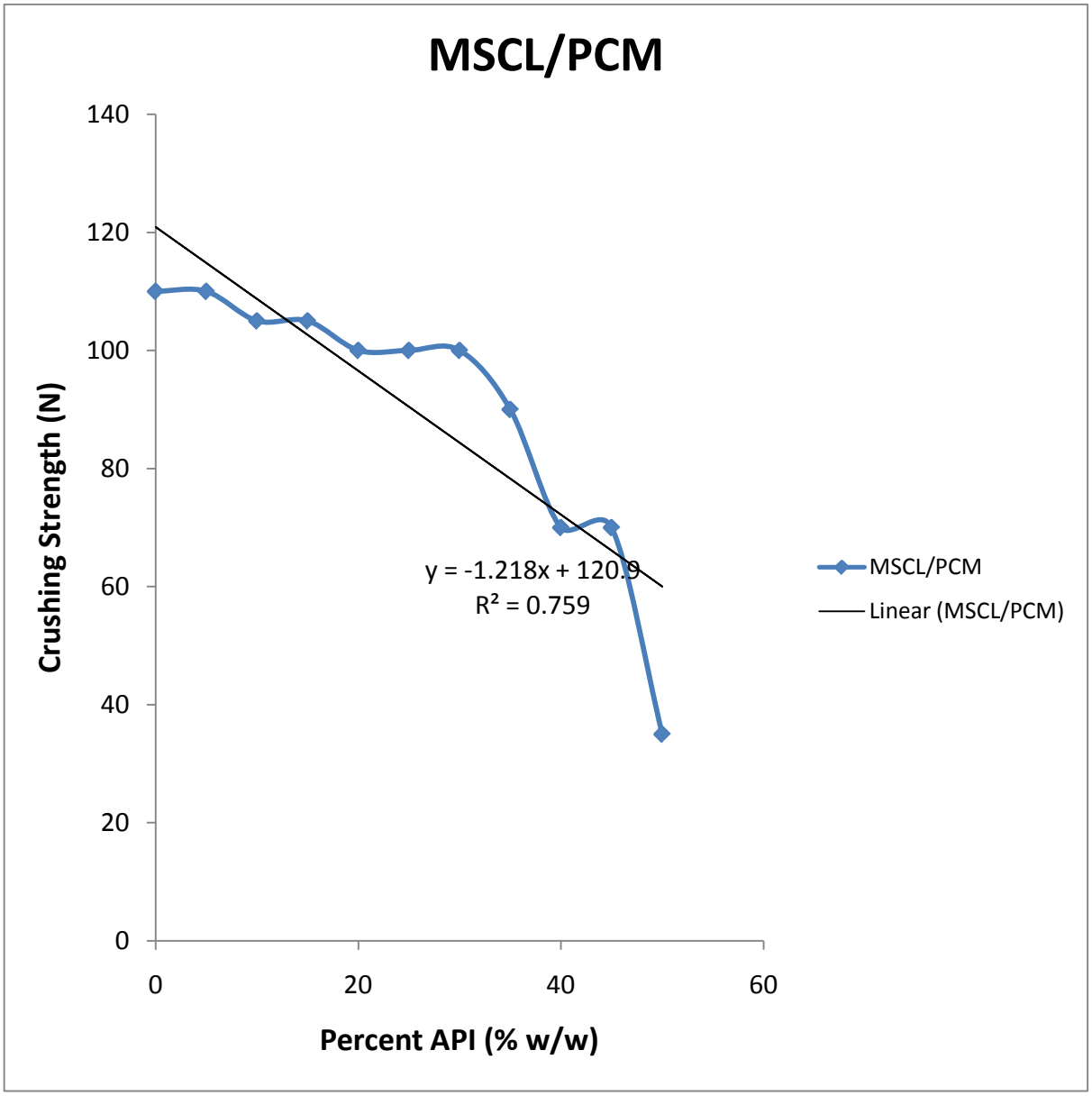

Figure 11 : Shows the effect of increasing percentage of paracetamol (PCM) on tensile strength ofMSCLcompacts.

Table 5 : Shows summary of tablet properties of compacts at the limiting in-take of the Active Ingredient.

Tablet	Model drug	Dilution capacity (%)	Tablet Hardness (N)	Friabilty (%)	Disintegration Time (Sec)	REMARK
MSCL/PCM	PCM	40	70	0.5	25	Good
		45	70	0.6	23	Good
MSCL/AA	AA	45	73	0.4	119	Good
		50	68	0.5	90	Good

NB: MSCL, PCM and AA represent microcrysatarcellac, paracetamol and ascorbic acid respectively.

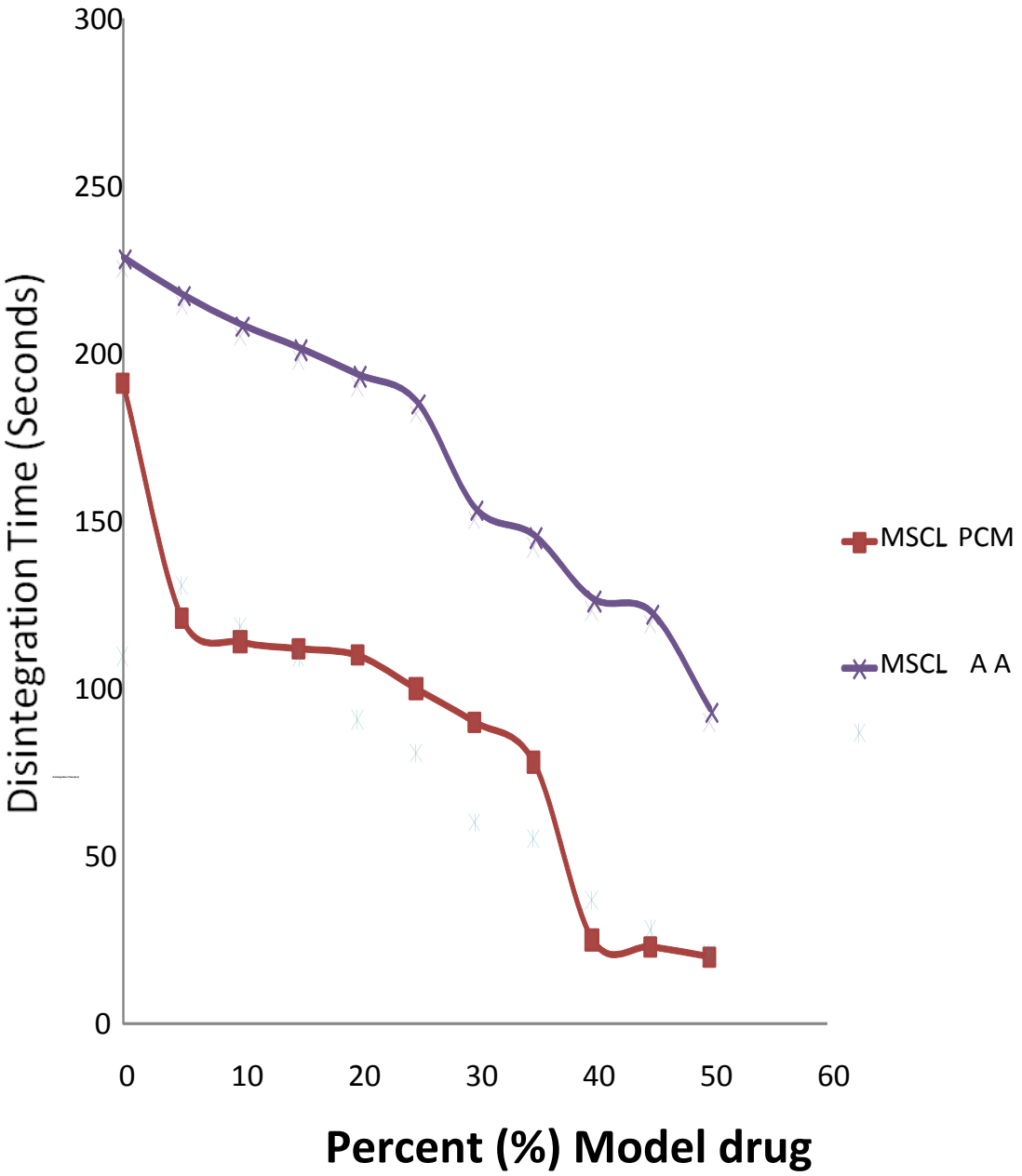

Figure 12 : Shows the effect of increasing the percentage of model drug \[Paracetamol (PCM) and Ascorbic Acid (AA)]on disintegration time of tablets formulated with MSCL.

Figure 13 : Percentage Drug Release vs Time (min). Absorbance: PCM.

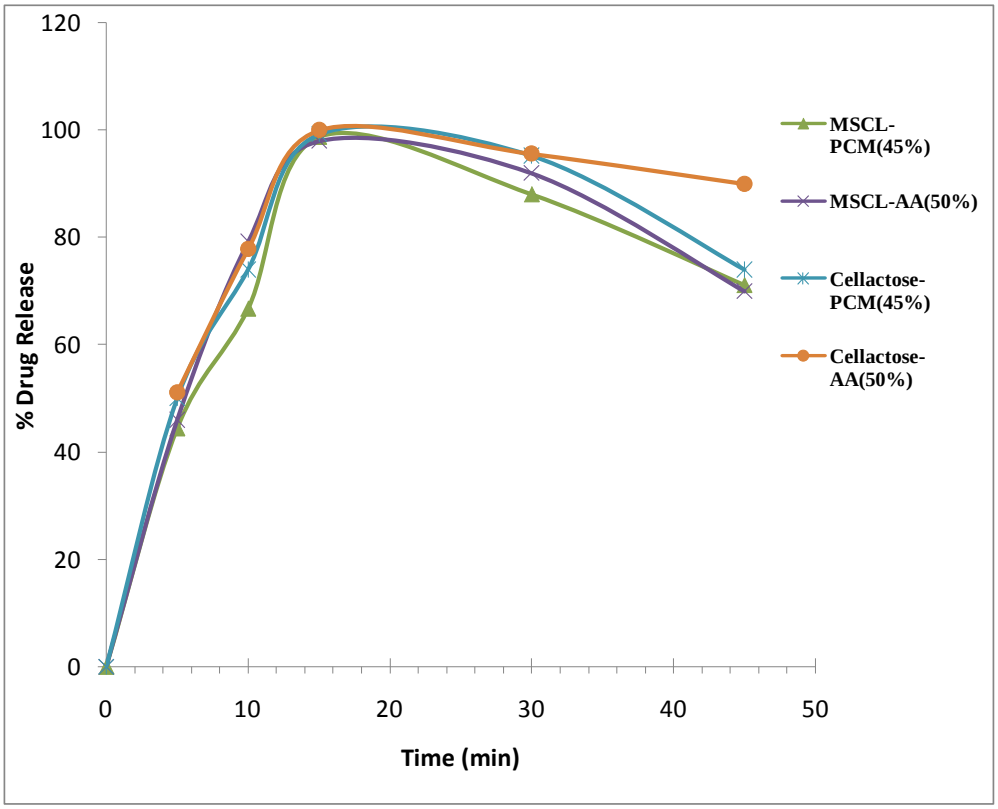

Table 6 : Dissolution rate constant (kD) obtained from Kitazawa equation.

	MSCL-PCM	MSCL-AA	Cellactose-PCM	Cellactose- AA
k_D (mg min^{-1})	7.5 x 10^{-3}	11.0 x 10^{-3}	9.3 x 10^{-3}	10.3 x 10^{-3}

The rate constant obtained from dissolution data presents following sequence of dissolution order:

k_D at t=10 min.

MSCL-AA (11.0 x 10^{-3})> Cellactose-AA(10.3 x 10^{-3}

Cellactose -PCM (9.3 x 10^{-3}) > MSCL- PCM (7.5 x 10^{-3})

a) Microcrystarcellac (MCTS 40 %: LMH 40 %: MCC 20 %)

i. Granular properties

Table 2 compares the granule properties of coprocessed MSCL (MCTS 40 %: LMH 40 %: MCC 40 %) with the direct physical mixtures of the same ratio, Starlac®, Cellactose® and MCC. The result illustrates an increase in flow properties of coprocessed MSCL over that of the direct physical mixture as reflected by flow rate 2.0 g/s, for the former and 0.45 g/s, for the later respectively. The corresponding angles of repose are 32° and 47.8° respectively. The compressibility indices as reflected in the table are: 13.4 % and 52 % respectively. All these results indicate improvement in both flow property and compressibility of MSCL after coprocessing over direct physical mixture of the same ratio. The coprocessed granules were restructured by sieving to remove the fine and granules greater than 250 μm. The MSCL granule distribution in percent cumulative retained oversize versus granule size in micrometer (Fig.1) shows that 100 % of the granules were within 90 – 250 μm range. The free flowing characteristics of MSCL could be attributed to this structured granule size range.

Fig.1 was an illustration of the granule size distribution as composed in the MSCL (MCTS 40 % : LMH 40 % : MCC 20 %). More than 50 % of the granules were greater than 250 μm and all the granules (100 %) were greater than 90 μm, this range of granule size distribution was responsible for the improved flow property over individual and the direct physical mixture of the primary excipients.

ii. Tablet properties (Placebo tablets)

The MSCL tablets (Fig.7) appeared smooth, free from chipping and lamination. This is an evidence of a good and acceptable tablet formulation.

MSCL was subjected to compressibility and compactibility studies. The material was compacted using a single punch Carver hydraulic hand press (model, C, Carver Inc. Menomonee Falls, Wisconsin, U.S.A) over a pressure range of 2.5 to 12.5 KN. Fig.4.23 compares the compressibility of MSCL with Starlac, Cellactose and MCC. The MSCL curve shows a nonlinear early part followed by progressive increase in compact density with pressure, and appears lower than the curves for the standard excipients this is due to low porosity of the former compare to the later. As the porosity approaches zero, plastic deformation may be predominant mechanism for all powder material (Heresy and Rees, 1971: York and Pilpel, 1972) Fig. 4.26 shows the result of the compactibility studies, it illustrates the relationship between compression pressure and radial tensile strength for MSCL. The curve is similar to Heckel plot, it has two portions, and the early part representing consolidation as a result fragmentation, and some degree of plastic deformation, followed by a linear portion illustrating the consolidation behavior as a result of plastic deformation.

iii. Friability of MSCL (Placebo tablets)

Fig.3 shows the effect of increasing compression pressure on the friability of MSL compacts. There is a direct relationship between tablet hardness and compression pressure. Friability declined with both increase in compression pressure and tablet hardness. It can be seen that as the compression pressure increases from 2.5 N to 12.5 N, friability also decreases from 1.25 % to 0.5 % for MSCL.

iv. Disintegration Time of MSCL (Placebo tablets)

The presence starch granules in MSCL are expected to impact disintegration property. The disintegration time is mostly influenced by tablet hardness. Fig.4 shows the effect of increasing compression force on disintegration time for MSCL, Starlac, Cellactose and MCC. Disintegration time increases with increase in tablet hardness which is proportional to the applied pressure. The DT for all the compacts of MSCL formed between compression force 2.5 N and 12.5 N ranges from < 2min. to 3 min. The corresponding values for Starlac and Cellactose are: all < 1 min., and < 1 min to 17 mim., respectively. The B.P.C (1988) specified standard for conventional tablet to be 15 min. MSCL with disintegration time of 3 min. can be regarded as having a good inherent disintegrant property.

v. Densification behavior of MSCL (Placebo tablets)

a. Plot of Heckel equation

The widely used and relatively simple equation is given by:

$$\ln 1/[1 - D] = kp + A$$

Where, D is the relative density of the compact, 1 – D is the pore fraction, and p is the pressure. 'A' and *'k'* are constants of Heckel equation. The parameter A is said to relate to low pressure densification by interparticle motion, while the parameter k indicates the ability of the compact to densify by plastic deformation after interparticle bonding. Fig. 5 shows the plot of ln 1/[1 – D] *vs* p for MSCL, Starlac®, Cellactose® and MCC. The plot of MSCL can be divided into three-phases, namely: 29 $MNm^{-2} < p <$ 58 MNm-2, 58 $MNm^{-2} < p <$ 116 MNm-2, and 116 $MNm^{-2} < p <$ 144 MNm^{-2}, each of which basically obeys the Heckel equation. There is nonlinearity in the first phase (early stage) at low pressure which suggests that MSCL undergo fragmentation and rearrangement before plastic deformation (Odeku and Itiola, 2007). Under low pressure ($p < 58$ MNm^{-2}) the compaction would mainly result in the elimination of voids among the loose particles through rearrangement, fragmentation and some degree of plastic deformation, leading to rapid densification of MSCL. On the second phase from ~58 MNm^{-2} to ~116 MNm^{-2} , however, plastic deformation of MSCL particles would be responsible for the densification of MSCL compact. The third phase from ~116 MNm-2 to ~144 MNm-2, here, following decompression, an expansion in tablet height is represented by increased tablet porosity.

Table 3 show values of the mean yield pressure, P_y; the relative densities D_o, D_A, and D_B for MSCL, Starlac®, Cellactose® and MCC. P_y, is inversely related to the ability of the material to deform plastically under pressure. Low value of P_y indicates a faster onset of plastic deformation (Odeku and Itiola, 1998). The P_y obtained for MSCL, Starlac®, Cellactose® and MCC are: 22.3 MNm^{-2}, 143 MNm^{-2}, 24.2 MNm^{-2} and 25 MNm^{-2} respectively. From the values of P_y stated above, MSCL shows faster onset of plastic deformation than Starlac®, Cellactose® and MCC. The yield value of MSCL reflects better densification at low pressure than Starlac®, Cellactose® and MCC. Shangraw *et al.*,(1981) explains that, a large value of slop (i.e., low P_y value) is an indication that the onset of plastic deformation occurs at relatively low pressure and *vise visa.* This analysis has been extensively applied to pharmaceutical powders for both single and multi-component systems (Duberg and Nystrom, 1986; Itiola, 1991). D_A, represents the total degree of densification at zero and low pressures (Paronen and Juslin, 1983; Mitreveji *et al.,* 1996), (Roberts and Rowe, 1985). Do, is used to describe the initial rearrangement phase of densification as a result of die filling. D_o is equal to the ratio of bulk density at zero pressure to the true density of the powder (Chowhan and Chow, 1981). The relative density, D_B, describes the phase of rearrangement of particles in the early stages of compression and tends to indicate the extent of particle or granule fragmentation. From Table 3 the

D_o values for MSCL, Starlac, and Cellactose are: 0.470, 0.413, and 0.298. These results show that MSCL is more densify during the die filling than Starlac®, and Cellactose®. The DB values for the same set of materials are: 0.162, 0.404, and 0.157. These results reflect the degree of fragmentation at low pressure in the following order: Starlac®>MSCL>Cellactose®. Khan and Rhodes, (1975) has reported some degree of fragmentation in MCC with increase in compression pressure. Doelker, 1988; Nystrom *et al.*, 1993 observed that high D_B values are caused by fragmentation while low D_B values are associated with plastic deformation.

b. Plot of Kawakita equation

Kawakita equation can be written as [Kawakita and Ludde, (1970/71)]:

$$P/C = 1/a\ P + 1/ab$$

Where, a and b are constants ('a' gives the value of the minimum porosity of the bed prior to compression while 'b', which is termed the coefficient of compression, is related to the plasticity of the material) and C is the volume reduction, i.e., $C = (V_o - V)/V_o$ (here V_o and V are initial volume and the volume after compression, respectively). The Kawakita equation indicates that p/C is proportional to the applied pressure p. Fig.6 shows the plot of p/C vs p for MSCL,"Starlac®, and Cellactose®. One can see that a linear relationship exists between p/C and p in the whole pressure range investigated at correlation coefficient (R^2 = 0.982), which indicates that the densification behavior of MCTS is consistent with prediction from the Kawakita equations. By best fitting of the experimental data to the equation above one obtains:

$$p/C = 1.64\ p + 26.73$$

Hence, by relating the two formulae above, the value of "a" is obtained as 0.610 and "b as 0.0613 (1/b = 16.3).

The Di (=1 – a) indicates the packed initial relative density of tablets formed with little pressure or tapping (Lin and "Chain, 1995). Table 4 shows the Di values for MSCL, Starlac®, and Cellactose® as: 0.390, 0.474, and 0.286, respectively. It can be seen that at low pressure MSCL tablet is better packed than Cellactose tablets, but less in packing relative to Starlac tablet. This result is not far from the fact that packing of a material with applied pressure is determined by deformation propensity.

Table 4 shows the values of 1/b (P_k) obtained for MSCL, Starlac®, and Cellactose® as: 16.3, 19.1, and 17.0 respectively. The reciprocal of b yields a pressure term, P_k, which is the compression pressure, required to reduce the powder bed by 50 % (Shivanand and Sprockel, 1992). The value of P_k gives an inverse measurement of plastic deformation during compaction process. The lower the value of P_k, the higher the degree of plastic deformation occurring during compression (Itiola, 1991). The pressure term P_k has been shown to provide a measure of the total amount of plastic deformation occurring during compression (Odeku and Itiola, 1998). Hence, from the results of P_k values, MSCL is more plastically deformed during compression than Starlac®, and Cellactose®.

vi. *Dilution capacity/potential*

Tablets formulated from MSCL (55 %) and PCM (45 %) as shown in Figure 11, were smooth, free from chipping and lamination. More so, tablets formulated from MSCL (50 %) and AA (50 %) were also characterized by the same good and acceptable tablet qualities.

Fig.10 and 11 Illustrates the relationship generated from the amount in percent (%) of API compressed with MSCL and the crushing strength. It can be seen that tablet strength declined with increasing amount of API until it reaches a point where the tablet strength, friability and the physical structure failed to meet the official standard. Table 5 showed the summary of the result of the dilution potential. MSCL was compacted with PCM and AA in predetermined percentages as model drug (API).One can see that MSCL was able to form acceptable compact with maximum of 45 % of the former (crushing strength is 70 N and friability, 0.6 %, disintegration time, 23 sec.), and with 50 % of the later (crushing strength is 68 N and friability, 0.4 %, disintegration time, 90 sec.). Hence, MSCL – PCM- 45 % and MCTS – AA – 50 % are both acceptable dilution capacity/potential. MSCL can therefore be used for formulating poorly compressible API, highly compressible, moisture sensitive API.

a. Disintegration Time MSCL- Model drug

Fig. 12 shows the declining disintegration time with increasing percentage of API. It can be seen that the DT of MSCL – PCM and MSCL – AA ranges between ~2.1min., down to ~0.42 min., for the former and ~3.8 min., down to 1.5 min., for the later respectively. One can see that the disintegrant properties of MSCL is more pronounced in the formulation containing poorly compressible and water insoluble API (PCM) than in formulation containing highly water soluble and moisture sensitive API (AA).

vii. *Brittle Fracture Index (BFI)*

Both MSL and MSCL possessed BFI values as 0.1 and 0.08 respectively (Theoretical value range is 0 – 1). BFI has been used as a measure of plastoelasticity of pharmaceutical powders. A low BFI value indicates the ability of the material to relieve localized stresses while a value approaching unit indicates a tendency of the material to laminate or cap.

The combination of plastic and brittle materials in both MSL and MSCL helped to reduce storage of elastic property. Lamination or capping is s normally a result of high storage of elasticity.

viii. *In-vitro Drug*

Fig.13 also illustrate the graphs of percentage (%) drug release versus time (min) for MSCL – PCM and MSCL-AA. The table 4.19 shows T90% to be 13min and 12min respectively, and 100% of the drugs were released from both formulations in 15 min. The dissolution rate constant (KD) for both formulations at 10min were calculated to be 7.5 x 10-3 mg min-1 and 11.0 x 10-3 mg min-1 respectively.

ix. *Statistics*

The P values obtained at 95 % confidence interval for MSL and MSCL sampled at 6 months interval were >0.05, hence, the mean of differences does not differ significantly.

The P value obtained for MSL-PCM paired with Cellactose-PCM was >0.05, the result was considered not significant.

The P value for MSCL-AA paired with Cellactose-AA was >0.05, the result was also considered not significant.

IV. Conclusion

The crushing strength for NTS, ATS and MCTS are: 30 N, 90 N and 100 N after 3 h of annealing and hydrolysis respectively, compressed at 6 metric units.

MSCL have improved functionality over direct physical mixture of the primary excipients. The compression pressure, required to reduce the powder bed by 50 % (onset of plastic deformation) P_y (yield value) are: MSCL (22.3 MNm^{-2})>Cellactose (24.2 MNm^{-2})>MCC (25 MNm^{-2})> Starlac (143 MNm^{-2}). The degree of plastic deformation occurring during compression (P_k) is in the following order: MSCL (16.3 MNm^{-2})> Starlac®(17 MNm^{-2})>MCC (18.6 MNm^{-2})>Cellactose® (19.1 MNm^{-2}). From these two parameters (Py and Pk), MSCL has been established to be more superior to the three standard excipients namely: Starlac, Cellactose, and MCC.

The dilution potential obtained for MSCL compacted with paracetamol (PCM) and ascorbic acid (AA) as active drug (API) are: 50 % AA with MSCL, 45 % PCM with MSCL. MSCL is superior in functionality than Starlac, Cellactose and MCC. The hardness of MSCL containing 45 % PCM, is 70 N; MSCL containing 50 % AA, 68 N. MSCL can be used to formulate softer tablet of both poorly compressible API and moisture sensitive API.

Table 4.18 and 4.19 summarized the formulation studies on MCTS, MSL, and MSCL in PCM and AA tablets. All the formulations released 100% of its active ingredient in 15min, and it can be seen from the table that the T90% ranges from 12-14min for the formulations, and they compared favourably with Cellactose® and much better than Starlac ®.

From the table 4.18, the rate constants obtained from dissolution data presents the following sequence of dissolution order: KD at t = 10min. MSCL – AA (11.0 x 10^{-3} mg min^{-1}) > Cellactose – AA (10.3 X 10-3) Cellactose – PCM (9.3 x 10^{-3} mg min^{-1}) > MSCL – PCM (7.5 x 10^{-3}mg min^{-1}).

MSCL performed better than MSL, Starlac ®, and rated equal with Cellactose® in PCM and AA tablet formulations in terms of functionality. It can be used to formulate low dose up to 225 mg poorly soluble and poorly compressible API (i.e., 45 % of tablet weight) in which PCM represents the class of drug. Moreover, it formed better tablet with low dose up to 250 mg poorly compressible, highly soluble and moisture sensitive API (i.e., 50 % of tablet weight) this class of drug is represented by AA.

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23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw th

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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