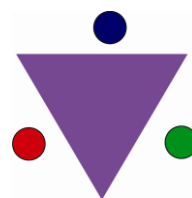


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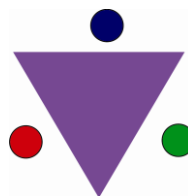


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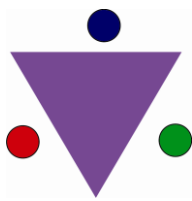
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BEHAVIORAL AND BIOCHEMICAL STUDIES OF SEEDS OF *CORIANDRUM SATIVUM* IN VARIOUS STRESS MODELS OF DEPRESSION

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ABSTRACT

Depression is one of the most common and costly brain diseases; there is a need to develop more effective medications to treat this devastating disorder. Among Indian population there is an increasing demand for herbal drugs due to their low side effects. Hence the plants are the good candidates for evaluation of antidepressant activity. The aim of present work was to investigate the antidepressant activity of seeds of *Coriandrum Sativum* using various behavioral models of depression. For this purpose Behavior tests like Forced swimming test and Tail suspension test were used. And also Biochemical estimation of Monoamine oxidase-B enzyme activity was carried out. Both extract of *Coriandrum Sativum* shows significant antidepressant effect and also inhibit the Monoamine oxidase –B enzyme. Taking together, the finding in the current study shows that extracts of seeds of *Coriandrum Sativum* displays a behavioral profile consistent with an antidepressant like action. Further research should be aimed to isolating the active principle responsible for antidepressant activity and exploring the mechanism by which it produce a antidepressant effect.

Keywords: Depression, Forced swimming test, Tail suspension test, Monoamine oxidase-B, antidepressant activity.

INTRODUCTION

Depression is one of the most prevalent psychopathologies in the world. Conventional antidepressant treatment has many limitations such as some are slow to take effect, side effect profile limiting compliance and there are also a large number of treatment resistant patients.¹ Such a profile has necessitated new therapeutic strategies in offering faster onset of action and augmenting the therapeutic actions of currently existing antidepressants and thus getting a greater

efficacy in a larger proportion of patients. *Coriandrum Sativum* L. has been recommended for relief of insomnia in Iranian traditional medicine and also brain tonic in Ayurveda². On the other hand various plants have been recommended brain tonics viz. *Withania somnifera* (Ashwagandha)³. *Asparagus racemosus* (Shatavari)⁴. *Bacopa monniera* (Brahmi) were evaluated for their antidepressant effect⁵. However, no pharmacological activity of *Coriandrum Sativum* have yet evaluated for its antidepressant effect. As the coriander seeds were evaluated for anxiolytic effect⁶. And the present work was undertaken to determine the antidepressant effect of aqueous extracts

and fatty oil of coriander seeds. The two models are used for present study i. e. forced swimming test and Tail suspension test. Both models reflect a state of despair which can reduce by several agents which are therapeutically effective in human depression⁷. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.⁷ It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. Measurement of immobility time was carried out by observing the motor activity of mice, which were placed in a pool of water.

MATERIAL AND METHOD

Seeds of *Coriandrum Sativum* were collected from local market in Sangli. The seeds of *Coriandrum Sativum* identified by the Department of Botany at Deccan Education Society's Willingdon College, Sangli.

Preparation of aqueous extract

Air dried seeds was homogenized to a fine powder. Hundred grams of powdered coriander was infused in 500 ml cold distilled water for 24 h, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. The extract was filtered, then concentrated over the water bath and brought to dryness under vacuum⁶. The yield of the extract was 6.5%.

Preparation of Fixed oil extract

The seeds of the *Coriandrum Sativum* were ground in mixer. The 100gm of powdered

material was macerated with 500ml of Diethyl ether for 2 hrs. The solvent was evaporated.^{8,9}. The yield of the extract was 1.02% w/v. The toxicity study was carried out according the test procedure described in OECD Guideline (425) to estimate acute oral toxicity of drug.¹⁰ from the result obtained by toxicity study, it was concluded that the drug extracts are safe and non toxic for use.

Screening methods

Male Swiss mice weighing 25gm were used. The animals were kept under normal light-dark conditions and at a constant temperature. The animals had free access to standard laboratory food and water. The mice were divided three groups, six animals in each group. One group served as control and received distilled water. And other two received 200 mg/kg & 400 mg/kg doses of Aqueous extract of *Coriandrum Sativum* for Forced swimming test and 300mg/kg & 600mg/kg for Tail suspension test. Aqueous extract of *Coriandrum Sativum* was suspended in distilled water, immediately prior to use and given as orally dosage. For the Diethyl ether extract, 0.4 ml/kg, 0.6 ml/kg given as orally for Forced swimming test and 0.6ml/kg & 0.8 ml/kg for Tail suspension test.

Forced swimming test

After 14 day administration of drug, Measurement of immobility time was carried out by observation the motoric activity of the mice, which were placed in a pool of water. A glass cylinder, 25 cm in diameter, height 23 cm was filled with water to a height of 12 cm. The temperature of water was 23+₋ 1⁰ c on the day of testing, after 30 min of dosing animal were subjected to test. Measurement was carried out for six min. The first two minute the animal was allowed to adjust to

the new condition after this two minute, the immobility time that alternated with conditions of enhanced motor activity was measured. Immobility time was measured with a stopwatch for the next four minute¹¹.

Tail suspension test

The tail suspension test was the second method for assessing the antidepressant effect of the extract. Thirty minutes after the single drug or vehicle injection, mice were subjected to the test.

A cord of about 50 cm in length was stretched between two mental tripods at a height of ca 70 cm, to which the mice were attached by the tail with sticky tape. After the initial period of vigorous motor activity, the mice become still and the immobility time was measured with a stopwatch, for a total duration of 4 minutes¹²

Statistics

For quantitative data statistical analysis was initially performed by using a one way analysis of variance (ANOVA).

Biochemical estimation

Preparation of brain Monoamine oxidase¹³

Rats (n=12) were decapitated and allowed to bleed. The brains were removed as quickly as possible, placed on a filter paper and their weights determined. All subsequent procedures were performed at 0–4 °C. The brains were rinsed thoroughly in cold saline (0.9% NaCl), then homogenized in 4 volume (w/v) of 0.25 M sucrose, 0.1 M sodium phosphate buffer (pH 7.4) in a Teflon glass homogenizer. The homogenates were centrifuged at 600×g for 10 min. The supernatant fraction was divided into 3-ml portions in small screw-cap vials and kept frozen for later assaying of MAO.

Kinetic assay of MAO-B¹³

All assays were carried out in triplicate at 30 ± 0.1 °C in 0.1 M sodium phosphate buffer, pH 7.4. Results are mean values \pm standard deviation of the mean. MAO-B activity was measured using benzylamine as substrate as follows: Mitochondrial protein (100 μ l, 600 μ g protein) in 0.1 M sodium phosphate buffer, pH 7.4 (total assay volume, 3 ml) was incubated in cuvette (pathlength 10 mm) in Ultramicroscopy and 1000 nm wavelength in UV/visible Spectrophotometer. The reaction was started by addition of benzylamine (25 μ l of 1 mM solution in water) and the progress of the reaction (formation of benzaldehyde) was monitored at 250 nm. Initial velocities as $\Delta A/\text{min}$ were measured from the time scanning of the reactions at 250 nm, ϵ ($\text{M}^{-1}\text{cm}^{-1}$) 12500. The maximum velocity was expressed as $\mu\text{mol/mg protein per min}$ ¹³.

RESULT AND DISCUSSION

Forced swimming test and Tail suspension test are currently most widely used models of animal depression¹¹. And has been validated for use with rat and mice. The indices of depression in these models were “Immobility Time” Shorter immobility time, stronger antidepressant effect.

In present study, two doses 200 mg/kg, 400 mg/kg of Aqueous extract and 2 ml/kg, 4 ml/kg of Diethyl ether extract have been administered for 14 successive days in FST model (Table no. 1). While 300 mg/kg and 600 mg/kg of aqueous extract and 6 ml/kg, 8 ml/kg of Diethyl ether extract of seeds of *Coriandrum Sativum* have been administered for 14 successive days in TST (Table no.2). The efficacies of the extracts were found to be comparable to fluoxetine (SSRI) and Imipramine (Tricyclic antidepressant). While comparing the results obtained by above two models,

which have been used different stress situations to induce states of terror and despair, it can be observed that the Diethyl ether extract i. e. fatty oil shows more potent effect on reduction of immobility time than aqueous extract (Table no. 3).

According to the M. Emamghoreishi et al, the aqueous extract of coriander seed caused a dose dependent general reduction of the spontaneous activity in pentobarbital-induced sleeping time, decreased general locomotor activity and neuromuscular coordination⁶. Present study also supports the above findings. But fatty oil of seeds of *Coriandrum sativum* did not show significant change in locomotor activity of mice as compared to control. So it did not produce any motor effect. It is confirmed the assumption that the antidepressant effect of fatty oil of seeds of *Coriandrum Sativum* is specific.

Depression is related to alterations of the monoamine oxidase. MAO-A and MAO-B are biochemical markers of depression. The conversion of Benzyl amine to benzaldehyde has been shown to occur readily in tissue homogenates and to be dependent upon monoamine oxidase activity. The reaction can be followed conveniently in a spectrophotometer and has been developed in to simple and rapid method for the assay of monoamine oxidase¹⁴. The results were expressed in % of control. In the present study, the Aqueous extract of seeds of *Coriandrum Sativum* showed marked decreases MAO-B activity with time (figure no. 1 & 2). Hence aqueous extract of seeds of *Coriandrum Sativum* showed antidepressant like activity probably by inhibiting MAO enzyme, thus increased monoamine levels of brain.

CONCLUSION

Taking together, the finding in the current study shows that extracts of seeds of

Coriandrum Sativum displays a behavioral profile consistent with an antidepressant like action.

1. In the psychopharmacological evaluation, the extracts of seeds of *Coriandrum Sativum* tested in forced swimming test & Tail suspension test, shows significantly antidepressant like action.
2. On the other hand, it was observed that Diethyl ether extract of seeds of *Coriandrum Sativum* showed more significant antidepressant effect than that of Aqueous extract when statistically significant difference compared to the control group of animal.
3. MAO-B is one of the biochemical marker of depression, antidepressant drug achieve their therapeutic effect through reversing alteration of this markers. Aqueous extract of seeds of *Coriandrum Sativum* showed antidepressant activity probably by inhibiting MAO enzyme.
4. By reviewing all the observations of FST & TST, it was indicated that both the extract of *Coriandrum Sativum* seeds have antidepressant like effect similar to that of Imipramine & Fluoxetine, therefore, it will be used in clinical practice.

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Table 1: Effect of aqueous extract and Diethyl ether extract of seeds of *Coriandrum Sativum* on immobility period in FST

Sr.no.	Drug treatment for 14 days / oral	No.of animals	Dose	Immobility time(s) (mean \pm SEM)
1	Control(distilled water)	6	10 ml	192 \pm 11.9
2	Fluoxetine	6	20 mg/kg	157 \pm 17.55
3	Imipramine	6	15 mg/kg	129.66 \pm 11.9
4	AECS	6	200 mg/kg	102 \pm 20.49
5	AECS	6	400 mg/kg	89 \pm 10.59
6	DEECS	6	2 ml/kg	83 \pm 13.8
7	DEECS	6	4 ml/kg	70.66 \pm 11.8

AECS = Aqueous extract of seeds of *Coriandrum Sativum*

DEECS = Diethyl ether extract of seeds of *Coriandrum Sativum*

Statistical analysis of data was carried out by one way ANOVA followed by Dunnett t- test.

Values are expressed as mean \pm SEM, P<0.001 is considered as criterion of significance.

P<0.01 as compared to control

Table 2: Effect of aqueous extract and Diethyl ether extract of seeds of *Coriandrum Sativum* on immobility period in TST

Sr.no.	Drug treatment for 14 days / oral	No.of animals	Dose	Immobility time(s) (mean \pm SEM)
1	Control(distilled water)	6	10 ml	188 \pm 6.75
2	Fluoxetine	6	20 mg/kg	109.16 \pm 25.48
3	Imipramine	6	15 mg/kg	150.16 \pm 6.49
4	AECS	6	300 mg/kg	102.66 \pm 31.86
5	AECS	6	600 mg/kg	86.5 \pm 8.26
6	DEECS	6	6ml/kg	115.5 \pm 21.14
7	DEECS	6	8ml/kg	93.83 \pm 14.49

AECS = Aqueous extract of seeds of *Coriandrum Sativum*

DEECS = Diethyl ether extract of seeds of *Coriandrum Sativum*

Statistical analysis of data was carried out by one way ANOVA followed by Dunnett's- test.

Values are expressed as mean \pm SEM, P<0.001 is considered as criterion of significance.

P<0.01 as compared to control

Table 3: Comparison between effect of aqueous extract and diethyl ether extract of seeds of *Coriandrum Sativum* on TST and FST

Treatment	Dose	FST		Dose	TST	
		Immobility time(s)	% change		Immobility time(s)	% change
Control	-	192 ± 11.9	-	Control	188 ± 6.75	-
Aq. Extract	200 mg/kg	102.33 ± 20.49	46	300 mg/kg	102.66 ± 31.66	45
Aq. Extract	400 mg/kg	89.16 ± 10.59	54.5	600 mg/kg	86.5 ± 8.2	53
Diethyl ether extract	2 ml/kg	83 ± 13.8	56.7	6 ml/kg	115.5 ± 21.14	72.5
Diethyl ether extract	4 ml.kg	70.66 ± 11.8	63.19	8 ml/kg	93.83 ± 14.49	94.17

FST: forced swimming test, TST: Tail suspension Test

Figure 1: Spectrophotometric Determination of MAO-B activity of aqueous extract of Seeds of *Coriandrum Sativum* in vitro

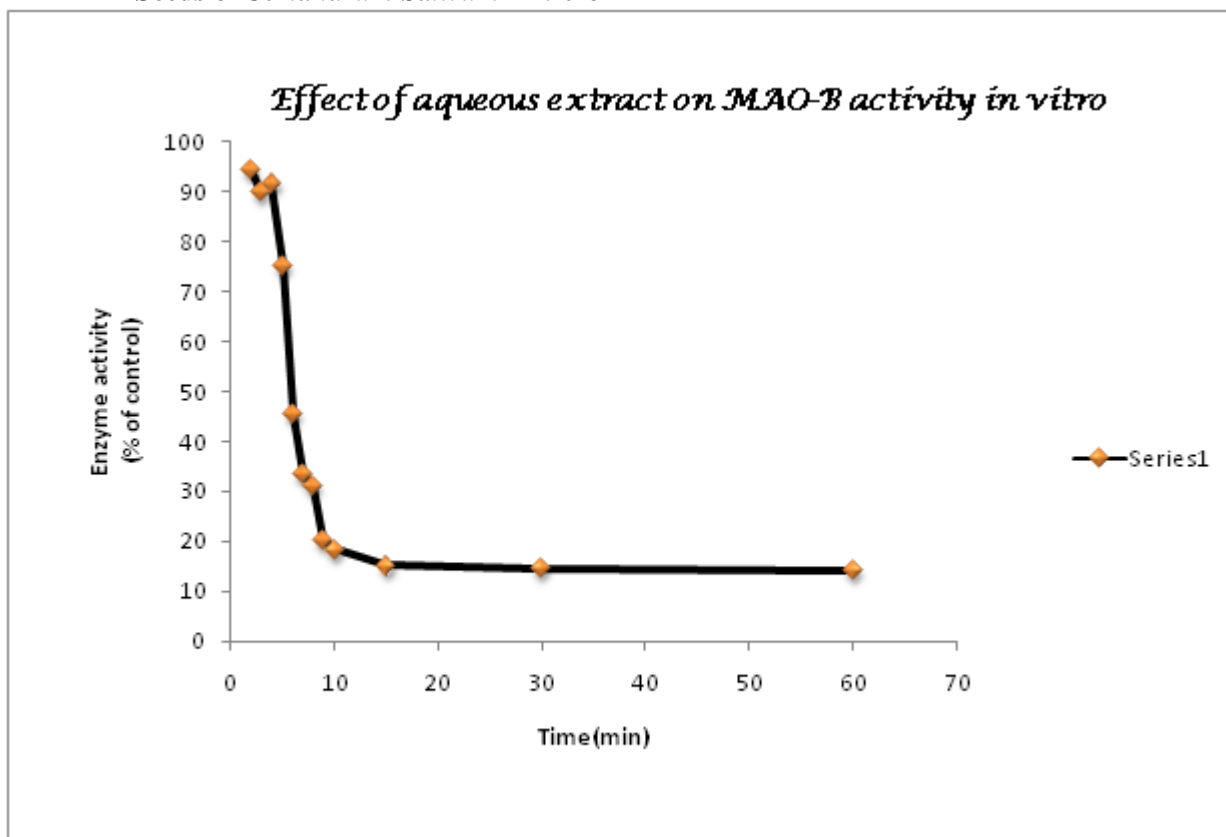
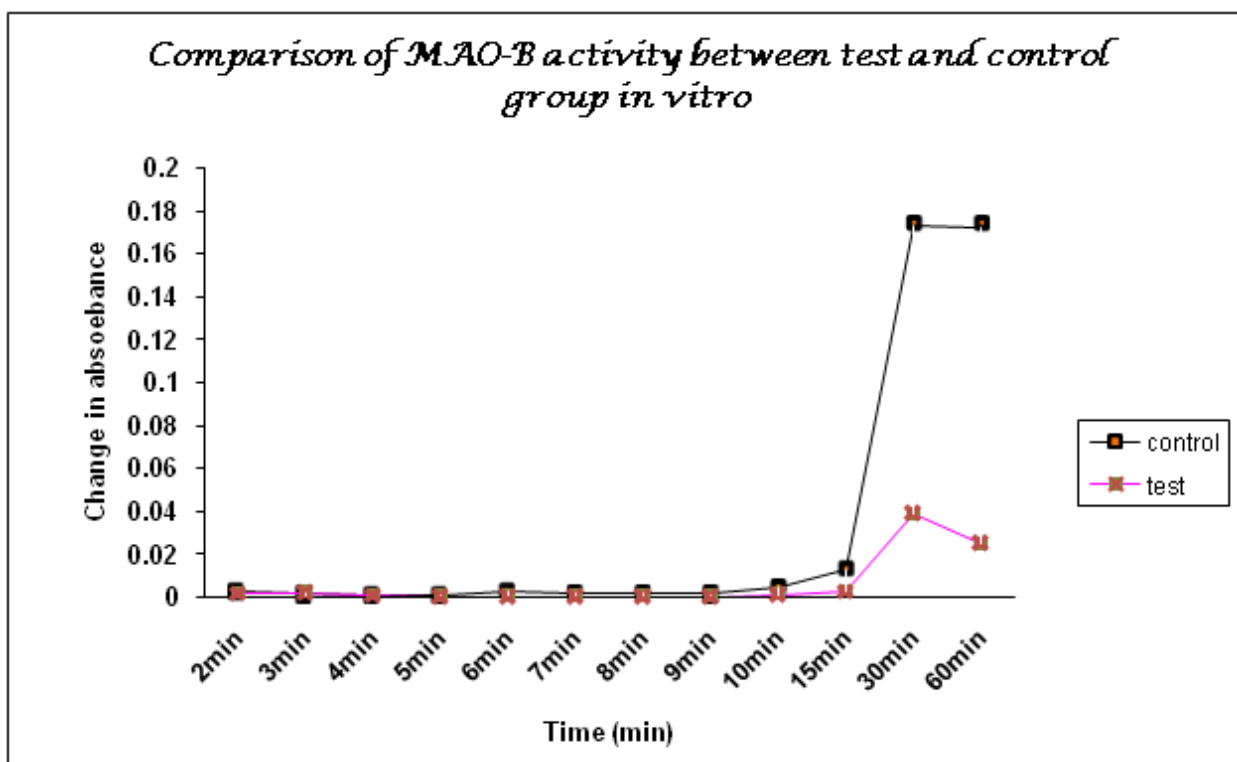


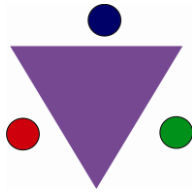
Figure 1. Shows Activity of Monoamine Oxidase enzyme was decreases with time

Figure 2: Comparison of MAO-B activity between test and control group in vitro



Test = Aq. extract of seeds of *Coriandrum Sativum*.

Figure 2 Shows increase in absorbance in control group due to increase in enzyme activity with time.



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IMMEDIATE EFFECTS OF PROPHYLACTIC KNEE TAPING AND BRACING ON PROPRIOCEPTION AND DYNAMIC BALANCE IN ASYMPTOMATIC AMATEUR ATHLETES- A RANDOMIZED CROSS-OVER TRIAL

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ABSTRACT

Purpose of the study: To compare the immediate effects of prophylactic taping and bracing on proprioception and dynamic balance on asymptomatic knee in amateur athletes.

Methods: 36 subjects of both sexes (mean age group was 22.3 ± 1.8) were included in the study. Inclusion criteria were minimum proprioceptive degree error: 3^0 using universal goniometer, full and free range of motion of lower extremity. They were randomized into two groups, group A and group B using block randomization, with 18 subjects in each group. Their proprioception (universal goniometer) and dynamic balance (SEBT) were assessed in both the knees, and the values were noted by the observer. The subjects underwent, either taping or bracing in both the knees and their proprioception and dynamic balance were assessed again. Followed by 24hrs of rest, cross-over of subjects to other technique were performed.

Results: The result showed that there is significant improvement in both proprioception and dynamic balance with taping and bracing. Application of tape and brace on right knee showed a significant reduction in proprioceptive error. The amount of decrease in error was $3.94^0 \pm 1.7^0$ and $5^0 \pm 1.9^0$ with bracing and taping respectively. The same when analyzed for left side the error significantly reduced to $3.92^0 \pm 1.7^0$ with bracing and $4.89^0 \pm 1.5^0$ with taping. Tape and brace have shown equal effect on proprioception. With taping and bracing there is statistically significant increase in dynamic balance ($p < 0.001$) in all the direction of SEBT for bilateral knee.

Conclusion: Bracing and taping were effective in improving the proprioception and dynamic balance of the amateur athletes.

KEY WORDS: Taping, Bracing, Dynamic Balance, Proprioception, SEBT.

INTRODUCTION

Knee is the most vulnerable site for injuries sustained by the athletes, who engage in sports that demands frequent change in the direction of the body movement and rapid acceleration and deceleration. Moreover

knee injuries can significantly affect performance and result in the lost practice and game time and they can lead to the development of chronic knee instability and pain.¹ Because of the high prevalence of these injuries, many organized sports association have implemented prophylactic measures in an attempt to decrease the incidence and prevalence of injuries,

however controversy continues to exist in regard to the best method of treatment and prevention of these injuries.^{2,3}

Proprioception and balance are two of the essential components that play an important role in avoiding the injury rates.⁴ Proprioception has been indicated to coordinate the following movement variables: positioning, force, and velocity. Because of its importance for coordinating sophisticated movements, proprioception is even more crucial for the skill-demanding movements (e.g., tennis playing) than daily movements.^{5,6} The effect of proprioception on the sports and its difference between patients and healthy adults has been widely tested. However, current knowledge provides insufficient insight into the effect of experience within an amateur athletic population.⁴

Measures of proprioception characteristically have high variability between different measurement techniques and also between subjects within the same measurement technique, one making objective comparisons of baseline joint position sense (JPS) scores difficult between subjects.⁷

Dynamic balance is required for normal daily activities, such as walking, running and stair climbing. Sports activities also require proper balance control. The visual, somatosensory, and vestibular systems all contribute to the maintenance of balance and may be adversely affected by musculoskeletal injury, head trauma, disease, or aging. These influences on the visual, somatosensory, and vestibular systems might decrease a person's ability to perform dynamic activities and, thus, impede normal daily functioning. Quantification of balance, or postural control, is often necessary to assess the level of injury or ability to function in order to initiate an appropriate plan of care.⁸

Squatting exercises are commonly included in lower-extremity rehabilitation programs in an effort to improve strength, balance, and neuromuscular control. Recently, a more complex squatting task to train and assess lower-extremity balance and neuromuscular control has been reported. The Star Excursion Balance Tests (SEBTs) are a series of unilateral mini squats performed while attempting with the opposite leg to reach as far as possible in a given direction.⁹

Proprioceptive training, star excursion balance exercise, taping and bracing are commonly employed in sports to prevent injury and re-injury. Athletic taping and bracing can prevent injury or facilitate injured athletes return to competition. In general the tape will limit the abnormal or excessive movement of the sprained joint while also providing support to the muscle that the sprain has compromised. Many clinicians attribute the value of taping to the enhanced proprioceptive feedback that the tape provides the athletes during performance.¹⁰ The literature shows taping and bracing has positive effect on proprioception but comparing among these which is better is not been studied. The aim and objective of the study was to compare the immediate effects of prophylactic taping and bracing on proprioception and balance on asymptomatic knee in amateur athletes.

METHOD

Study was approved by ethics committee of K.M.C Mangalore. Study was conducted at Kasturba Medical College, Mangalore. The inclusion criteria were Minimum proprioceptive degree error: 3^0 using universal goniometer (pilot study) and subjects with full and free range of motion of lower extremity. Subjects were excluded if they had any neurological deficits of

lower extremity, history of lower extremity injury within 6 months and deformity of lower extremity / spine.

Demographic data of the participants were collected. Subjects were randomized into two groups, group A and group B using block randomization with 18 subjects in each group. Their proprioception (universal goniometer) and dynamic balance (SEBT) was assessed in both the knees, and the values were noted by the observer, the tester was blinded. The subjects underwent, either taping or bracing in both the knees depending on the block randomization and their proprioception and dynamic balance were –assessed again, Followed by 24hrs of rest, cross-over of subjects to other technique were performed. During the 24hrs rest period the subject were advised strictly not to undergo any form of training.

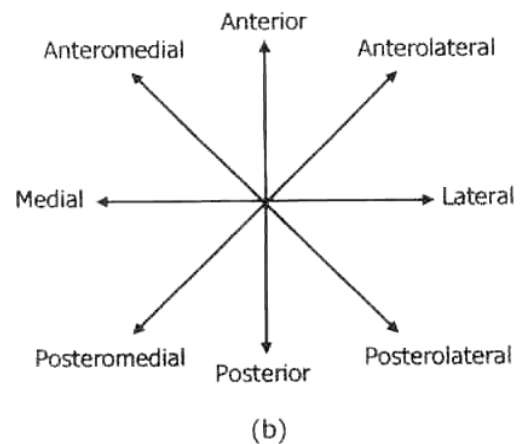
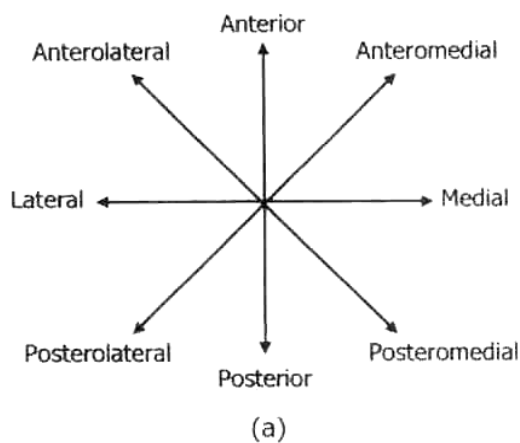
Proprioceptive Error Measurement Procedure

The subjects were asked to sit on a table, with their legs allowed to hang freely over the side of the table at a distance of 5cms to 10cms proximal to the popliteal fossa. The knee joint was palpated to place the goniometer, as the lateral joint line acts as the fulcrum (fig 1). The subject’s knee was then extended to 45°, passively and held for 10 seconds in order to memorize the position. Same procedure was repeated

with the subject blindfolded and the leg was again held for 10 seconds to facilitate memorizing the position by the subject. In the fourth step the subject still being blindfolded was asked to achieve or place his knee range of motion in the same range i.e. 45°, actively. The degree of error was hence noted by the observers, and the proprioceptive error was calculated.

Star Excursion Balance Test Procedure (SEBT)

The SEBT was performed with the participants standing in the middle of a grid formed by eight lines extending out at 45° from each other (fig 2). The participant were asked to reach as far as possible along each of the eight lines, make a light touch on the line, and return the reaching leg back to the center, while maintaining a single-leg stance with the other leg in the center of the grid. Participants were instructed to make a light touch on the ground with the most distal part of the reaching leg and return to a double leg stance without allowing contact to affect overall balance. The terminology of the excursion direction is based on the direction of reach in relation to the leg stance. When reaching in the lateral and postero-lateral directions participants reached from behind the stance leg to complete the task.



Participants were allowed to practice reaching in each of the eight directions six times to minimize the learning effect. Following a five minute rest period, participants performed three trails in each of the eight directions. They began with the anterior direction and progressed clockwise around the grid. After completion of the three trails of the eight directions and another five minute rest period, the test continued with the stance leg.

The investigator recorded each reach distance with the mark on the tape as the distance from the center of the grid to point of maximum excursion by the reach leg. At the conclusion of all trails, the investigator measured the distance of each excursion with a standard tape measure.

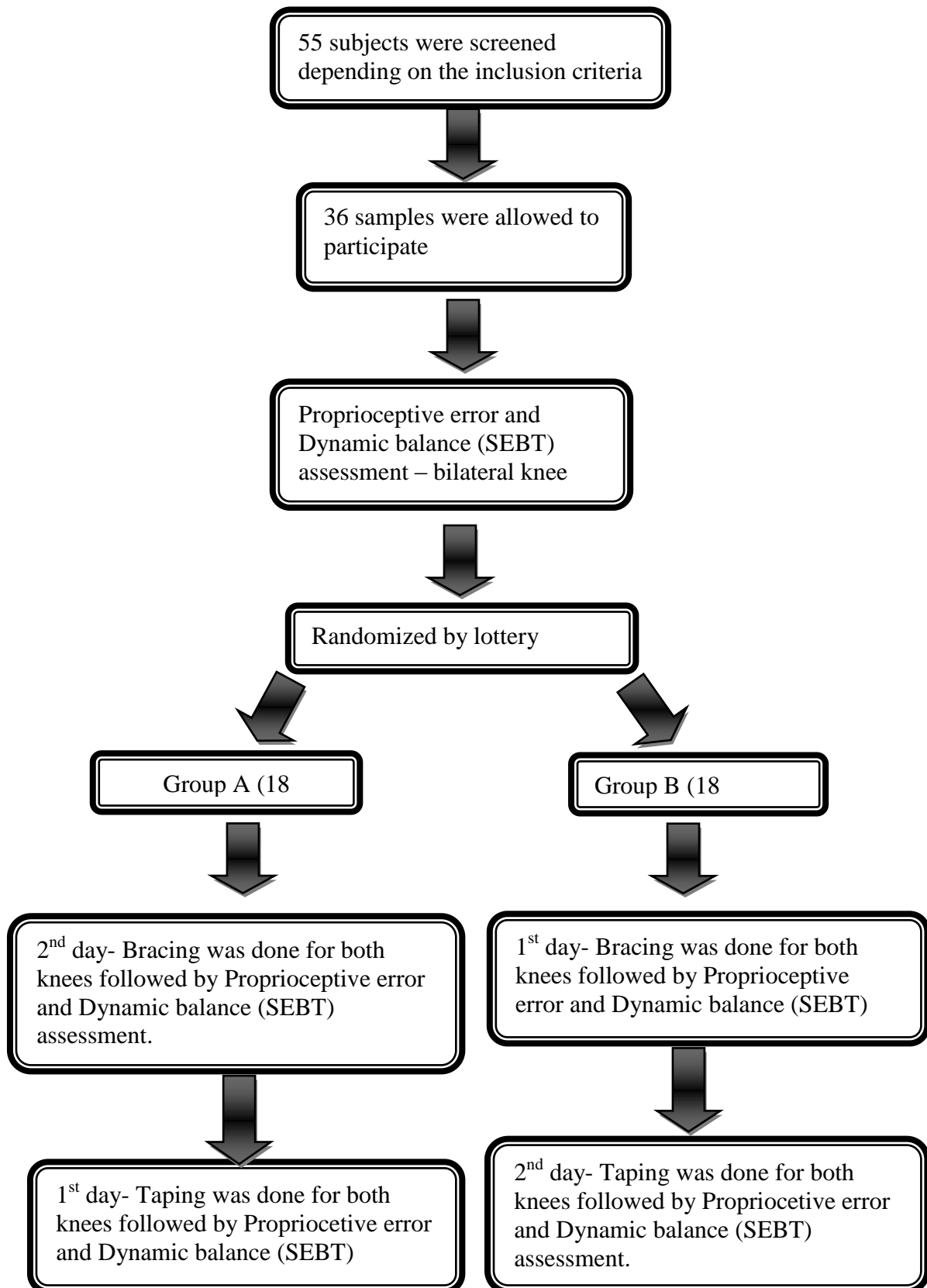
If the investigator felt the participants used:

- The reaching leg for a substantial amount of support at any time
- Removed his/her foot from the center of the grid or
- Was unable to maintain balance on the support leg throughout the trails

The trail would be discarded and repeated.¹¹

Taping Technique Knee Support: Diamond Wrap: Split both ends of the tape, forming four tails. Stretch the tails and apply firmly around the patella superiorly and inferiorly, interlocking the ends (fig 3). Close off with a strip of tape.¹²

Schematic flow chart of the procedure



RESULTS

In our study the numbers of samples included were 36, 28 were females and 8 males. The mean age group was 22.3 ± 1.8 . Taping and bracing has improved the proprioceptive and dynamic balance and comparison between them showed taping to be statistically better than bracing.

1. Comparison between bracing & taping and right knee & left knee

A. Proprioceptive error

Wilcoxon signed rank test showed statistical significant reduction in error in post bracing and taping in right as well as left knee. (Table 1)

Table-1: Comparison between right and left proprioceptive error using Wilcoxon signed ranks test.

Leg	Method		Mean \pm Std. Deviation	A difference in error	Wilcoxon Signed Ranks Test Z value	p value
Right	Post-Bracing AROM	Baseline P. Error	5.31 ± 1.9	3.94	5.185	p<0.001*
		Post P. Error	$1.36 \pm .93$			
	Post-Taping AROM	Baseline P. Error	5.31 ± 1.91	5.00	5.259	p<0.001
		Post P. Error	$.31 \pm 1.03$			
Left	Post-Bracing AROM	Baseline P. Error	5.56 ± 1.29	3.92	5.258	p<0.001
		Post P. Error	1.64 ± 1.22			
	Post-Taping AROM	Baseline P. Error	5.56 ± 1.29	4.89	5.260	p<0.001
		Post P. Error	$.67 \pm .89$			

* p<.001 – highly significant (HS)

Table-2: Difference in proprioceptive error of right and left knee using mann-whitney test.

	Leg	Method	Mean diff. in error \pm Std. Deviation	Mann-Whitney Z value	P
Diff. Error	Right	Post-Bracing AROM	3.94 ± 1.77	2.177	.029*
		Post-Taping AROM	5.00 ± 1.95		
	Left	Post-Bracing AROM	3.92 ± 1.79	2.649	.008
		Post-Taping AROM	4.89 ± 1.56		

* p<.001 – highly significant (HS)

Table-2, showed decrease in right side error as 3.94 ± 1.7 in bracing and that of taping as 5 ± 1.9 . Similarly left side the error significantly reduced to 3.92 ± 1.7 in bracing and 4.89 ± 1.5 in taping. The Mann-Whitney test showed that there was significant difference between bracing and taping with respect to reduction in error, which was more in taping. Hence taping was significantly better and error reduction

in the right knee was more compared to the left knee.

B. Dynamic balance assessment

Table- 3 and 4 demonstrates statistically significant change and increase in the dynamic balance when compared to bracing in most of the directions and it showed better effect in the right knee than the left knee.

Table-3: Comparisons between bracing and taping for dynamic balance for right knee using Mann-Whitney test.

Leg		Method	Mean change ± Std. Deviation	Mann-Whitney Z value	P
Right	change in ANT	Post-Bracing Post-Taping	6.29 ± 5.99 7.85 ± 7.89	.99	.324*
	change in ALAT	Post-Bracing Post-Taping	5.208 ± 4.32 8.144 ± 5.07	2.28	.023
	change in LAT	Post-Bracing Post-Taping	5.425 ± 5.25 7.958 ± 5.34	2.24	.025
	change in PLAT	Post-Bracing Post-Taping	5.386 ± 7.62 6.533 ± 6.04	1.35	.178
	change in POST	Post-Bracing Post-Taping	5.947 ± 7.31 10.086 ± 11.42	2.44	.015
	change in PMED	Post-Bracing Post-Taping	4.892 ± 5.02 7.567 ± 8.33	1.37	.171
	change in MED	Post-Bracing Post-Taping	8.653 ± 9.41 9.217 ± 9.49	2.79	.005
	change in AMED	Post-Bracing Post-Taping	6.642 ± 5.76 9.475 ± 7.21	3.60	.000

* p<.324 – not significant (NS)

Table-4: Comparisons between bracing and taping for dynamic balance for right knee using Mann-Whitney test.

Leg		Method	Mean change ± Std. Deviation in cms	Mann-Whitney Z value	p
Left	change in ANT	Post-Bracing Post-Taping	4.50 ± 5.02 6.53 ± 5.45	3.17	.002*
	change in ALAT	Post-Bracing Post-Taping	3.803 ± 4.06 6.022 ± 4.20	1.60	.110
	change in LAT	Post-Bracing Post-Taping	2.964 ± 6.73 4.522 ± 6.67	1.53	.127
	change in PLAT	Post-Bracing Post-Taping	5.092 ± 10.15 7.814 ± 7.76	2.19	.028
	change in POST	Post-Bracing Post-Taping	5.228 ± 5.67 9.992 ± 10.17	2.33	.020
	change in PMED	Post-Bracing Post-Taping	4.128 ± 3.22 9.453 ± 6.82	2.51	.012
	change in MED	Post-Bracing Post-Taping	5.956 ± 5.99 11.156 ± 8.69	4.05	.000
	change in AMED	Post-Bracing Post-Taping	4.233 ± 5.95 6.306 ± 6.72	1.57	.117

* p<.001 – highly significant (HS)

DISCUSSION

Our study aimed at finding the immediate effects of prophylactic knee taping and bracing on proprioception and dynamic

balance in asymptomatic amateur athletes.

The result showed that there was significant improvement in both

proprioception and dynamic balance with taping and bracing.

Application of tape and brace on right knee showed a significant reduction in proprioceptive error. The amount of decrease in error was 3° and 5° with bracing and taping respectively. The same when analyzed for left side the error significantly reduced to 3° with bracing and 4° with taping. Tape and brace have shown equal effect on proprioception. The minimum error considered for proprioception is 1° using the electronic goniometer.⁴ In this study we used the universal goniometer, the minimum error established was 3° , which was found from our pilot study.

In the present study, taping and bracing showed significant improvement in proprioception. Earlier studies have done on the effects of knee bracing on the joint position sense of subjects with anterior cruciate ligament reconstruction. Results indicated a significant difference for the knee joint angle repositioning test with bracing. In addition, the present study had the same positive findings as McNair et al who reported a significant improvement in the proprioceptive performance of normal subjects who used a knee sleeve.¹³

As active repositioning relies on afferent feedback from both muscle and joint mechanoreceptors, and that this greater influx of neurological information to the brain and spinal cord tends to increase repositioning ability. The additional information from the muscle mechanoreceptors involved in the active movement and going to the brain requires more processing and increases the precision involved with joint repositioning. It would seem plausible that both Golgi tendon organs (GTO) and muscle spindle activity may be improved as a result of the compression to muscle following

application of tape or brace, as muscle spindle is a major receptor of movement. Mechanoreceptors respond specifically to extremes in range of motion and localized compressions. Also, the traction of the tape on the hair and skin provides sensory cues about orientation and position, thus improving joint position sense.¹⁴

The other outcome measure in this study was dynamic balance (SEBT). With taping and bracing there is statistically significant increase in dynamic balance, in all the direction of SEBT for bilateral knee. The increase in dynamic balance following taping and bracing would help athletes to enhance performance and reduce injury. The mechanism by which it is attained is by joint stability. Joint stability requires the interaction of three different subsystems – the passive (the bone, ligaments, fascia and any other non-contractile tissue such as discs and menisci), the active (the muscles acting on the joints) and the neural (central nervous system and nerves controlling, the muscles) subsystems. The most vulnerable area of a joint is known as the neutral zone, where little resistance is offered by the passive structures. Dysfunction of the passive, active or neural systems will affect the neutral zone and hence the stability of the joint. The size of the neutral zone can be increased by injury. Muscle strengthening and application of external support to the joint, decreases the neutral zone.¹⁵

When we compared taping and bracing, bracing restricted the anterior lateral, lateral, posterior, medial and anterior medial directions of right knee and anterior, posterior lateral, posterior, posterior medial and medial directions in the left knee. This can be because of braces that restrict the motion, which reduces the opposite-limb reach distances in the braced stance leg. Whether ROM is

limited by the brace, causing other segments in the kinetic chain to compensate and achieve necessary motion, is unknown. It is important to examine dynamic balance to gain insight into how braces affect motion in specific directions. Restricted motion in a single direction may affect one's performance or risk of injury and, therefore, whether a brace should be used for prophylactic purposes in a healthy athlete must be carefully considered.¹⁶

Our results revealed that taping and bracing have significant effect on proprioception and dynamic balance. Tape and brace have certain advantages and disadvantages over each other. So the use of either of them would also depend on these factors. Taping can limit the range of knee, thus offering protection from over stretch or impingement of non-contractile structures. However, the taping technique used by athletes and physiotherapists is often governed by personal preference, the experience of the person applying the tape, and a general "feel" as to the correct technique.¹⁷

Braces have advantages over tape in being self applied without needing the expertise of qualified personnel, convenient to apply and remove," reusable, readjust able, and washable. Also, skin problems are less common, especially among those athletes who have an allergic reaction to elastoplasts or zinc oxide. These readymade braces are of various materials, thus providing varying amounts of support and stability. The non rigid braces are often made of canvas or a neoprene-type material, which can easily be slipped on and off, some with additional lacing. A number of studies have established the role of braces in restricting the amount of movement.¹⁵

Though, as mentioned above bracing have its advantages over taping. Hence the

choice of use among taping and bracing would depend on sports specific needs. We measured the proprioceptive error with the universal goniometer, it was the limitation of our study. Before performing the star excursion balance test, the sports shoe used were also not being assessed.

The long term effectiveness of tape and brace were not studied, and there is a need to study the comparison between tapes and brace effectiveness in injured athletes and its sports specific use. Our study population was amateur athletes, studies are warranted to be done in elite groups and post fatigue. Also, studies are required to analyze the various taping techniques.

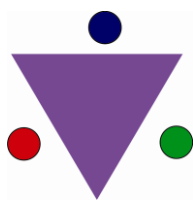
CONCLUSION

Bracing and taping were effective in improving the proprioception and dynamic balance of the amateur athletes.

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LECITHIN-MICROEMULSION BASED ORGANOGELS AS TOPICAL DRUG DELIVERY SYSTEM (TDDS)

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ABSTRACT

Most of the chemical entities that are being discovered are lipophilic in nature and have poor aqueous solubility, there by posing problems in their formulation into delivery systems. Because of their low aqueous solubility and high permeability, dissolution and/or release rate from the delivery system forms the rate-limiting step in their absorption and systemic availability. This frequently results in potentially important products not reaching the market or not achieving their full potential. Transdermal drug delivery provides the promising delivery system for those drugs. It provides better patient compliance by avoiding invasiveness, prolonging plasma drug level, bypass first pass effect, reduced side effects and easy termination of therapy. Interest in organogels has increased in a wide variety of fields including chemistry, biotechnology and pharmaceuticals. It is more reasonable to look for a carrier that interacts with the skin such that it allows various molecules to pass into the skin. In this paper, which follows a review on investigation of lecithin organogels as carriers for the transdermal transport of drugs having problems mentioned above. The general properties of lecithin organogels, method of preparation and its characterization have been discussed. The potential use of lecithin organogels for the transdermal transport of many therapeutic agents have also been discussed in the present review.

Keywords: Transdermal Drug delivery; Lecithin organogels; Release rate; Microemulsion-based gels; Rheology

INTRODUCTION

The skin is an exceptionally effective barrier to most drugs for therapeutic treatment. Very few drugs in therapeutic amount are permeated through skin such as nitroglycerine, scopolamine, nicotine, clonidine, fentanyl, estradiol, testosterone, Lidocaine, and oxybutinin [1]. Therefore,

the systems that make the skin more permeable and thereby enhance transdermal delivery are of great formulation interest. The strategies to deliver the medicament into the skin and for systemic circulation have been evolved. The extensive research has been reported on lipids as skin penetration enhancers [2-5]. Lipids in the form of vesicles such as liposomes, niosomes [6-8], ethosomes [9] and transfersomes [10] have been

evaluated. The lipid-based formulations have been in use since decades.

The importance of lipids has especially increased after realizing the utility of natural phospholipids. Lecithin, the natural biofriendly molecules are ubiquitous phospholipids that accounts for more than 50% of the lipid matrix of biological membranes. Soybean lecithin is an apolar organic solvent, on addition of water, forms an entangled dynamic network of long and flexible worm like multi-molecular aggregates termed as 'organogels' [11]. These are characterized by high viscosity and complete optical transparency. Lecithin organogels are emerging as carriers for drug molecules with diverse physicochemical properties including macromolecules [12]. Transdermal transport rates of scopolamine and broxaterol from lecithin organogels were faster than commercial patches [12].

With the advent of high throughput screening techniques, the discovery of biologically active molecules is taking place at a pace never seen before. Most of the chemical entities that are being discovered are lipophilic in nature and have poor aqueous solubility, there by posing problems in their formulation into delivery systems. Because of their low aqueous solubility and high permeability, dissolution and/or release rate from the delivery system forms the rate-limiting step in their absorption and systemic availability. More than 60% of potential drug products suffer from poor water solubility. This frequently results in potentially important products not reaching the market or not achieving their full potential. Pharmaceutical industry is quick in realizing the importance of solubility and dissolution rate in bioavailability and good deal of research has been done in this area. Currently a number of technologies are

available to address the poor solubility, dissolution rate and bioavailability of insoluble drugs [13]

Conventional Technologies

Conventionally used techniques [14] based on Noyes-Whitney equation [15] for enhancing solubility, dissolution rate and thereby bioavailability of insoluble drugs include buffered tablets, use of salts, solvates and hydrates, polymorphic forms, complexation, prodrugs, micronisation, solid dispersions and solvent deposited systems.

Newer Technologies

Newer and novel drug delivery technologies developed in recent years for bioavailability enhancement of insoluble drugs are described below.

Lipid Based Delivery Systems

Lipid emulsion technology [16], Self-emulsifying drug delivery system [17], Micro emulsion media as novel drug delivery system [18]

Microemulsion System

Microemulsions are four component mixtures composing of an oil phase, a water phase surfactant/s and a co-surfactant. The tendency towards formation of w/o or o/w microemulsions is dependent on the properties of the oil and the surfactant, the water-to-oil-ratio and the temperature. When a mixture of surfactant and co-surfactant is added to a biphasic oil-water system, a thermodynamically stable, optically transparent or translucent, low viscosity and isotropic mixture spontaneously forms. The transparency of these systems arises from their small droplets diameter (10-100 nm). Such small droplets produce only weak scattering of visible light when compared with that from the coarse droplets (0.5-100 μm) of traditional or standard macroemulsions such as emollient liquids, cream, lotions,

etc., Structurally, microemulsions have normal micellar solutions, reverse micelles, cores or droplets of water or oil, and, for some systems, even bicontinuous structures could solubilize large amounts of both oil and water soluble drugs within microemulsions.

There is rather confusing situation in the medical literatures, where the term “microemulsion” is indifferently used to indicate systems of presumably unlike structure (“true” microemulsions and miniemulsions). Some studies have compared the performance of different emulsified systems (macroemulsions, microemulsion, multiple emulsion and gel-emulsions) prepared with similar oils and surfactants for applications such as controlled drug release or drug protection. The surfactants used to stabilize such systems may be (i) Non-ionic (ii) Zwitterionic (iii) Cationic (iv) Anionic surfactants. Combinations of these, particularly ionic and non-ionic, can be very effective at increasing the extent of the microemulsion region [18,19].

Rational Approach to Drug Delivery To & Via Skin

There are three main ways to solve the problem of formulating a successful topical dosage formulation [20].

1. We can manipulate the barrier function of the skin e.g., topical antibiotics and antibacterials help a damaged barrier to ward-off infection, sunscreen agents and the horny layer protect the viable tissue from ultraviolet radiation and emollient preparations restore palatability to a desiccated horny layer.
2. We can direct drug to the viable skin tissue without using oral, systemic or other route of therapy.
3. The third approach uses skin delivery for systemic treatment. For example, topical drug delivery systems provide

systemic therapy for conditions such as motion sickness, angina and pain.

Dermatologists aim at five main target region—skin surface, horny layers, viable epidermis and upper dermis, skin glands and systemic circulation.

Emulsion-Gels as Topical Formulations

Transdermal and topical formulations are becoming increasingly important and their use in therapy is becoming more widespread. But the skin acts as a barrier to topically administered drugs. Attempts have been made to circumvent the skin barrier by several means, emulsion-gels being one such promising technique [21].

Organogels

The topical administration of drugs in order to achieve optimal cutaneous and percutaneous drug delivery has recently gained an importance because of various advantages such as ease of administration, non-invasive, better tolerated and compliance, local enhanced transdermal delivery, avoidance of local gastrointestinal toxicity, avoidance of first pass metabolism.

In search of a vehicle to deliver the medicament into the skin layer (cutaneous delivery) or through the skin and into systemic circulation (percutaneous absorption) and to target the skin, varied kind of formulation systems and strategies have been evolved. Amongst the many, the lipid-based formulations have been in use since decades. Pharmaceutically, lipid emulsions may allow the sustained release of drugs by sink mechanism [19].

The importance of lipids has especially increased after realizing the utility of phospholipids. The natural bio-friendly molecules which in collaboration with water can form diverse type of polymolecular/super molecular structure with retardant release in sustained release formulation [22].

The topical delivery has been attempted and made successful using a number of lipid based systems viz., vesicular systems [23], lipid microsphere, lipid nanoparticles [13], lipid emulsion [16], polymeric gels [24]. In a recent development, phospholipids in conjunction with some other additives have been shown to provide a very promising topical drug delivery vehicle i.e., lecithin organogel. Lecithin organogels (LOs) are thermodynamically stable, clear, viscoelastic, biocompatible and isotropic gels composed of phospholipids (lecithin), appropriate organic solvent and a polar solvent [25]. Lecithin organogel, the jelly like phase consists of three dimensional network of entangled reverse cylindrical (polymer like) micelle, which immobilize the continuous or macroscopic external organic phase, thus turning liquid into a gel [22].

The formation of three-dimensional network in the organogel is the result of transition at the micellar level in a low viscous network liquid consisting of lecithin cause micelles in non-polar organic liquid [26]. This spherical reverse micellar state of lipid aggregates, twins on to form elongated tubular micelles with the addition of water, and subsequently entangle to form a temporal three dimensional network in the solution bulk. The latter serves to immobilize the external organic phase, thus producing a gel form or the jelly like state of the initial non-viscous solution. However, the transparency and optical isotropy of the organogel remain as before. For this reason, these systems are often called as polymer like micelles and are also termed as living or equilibrium polymer, worm like or thread like micelles [22].

Advantages of Organogels as Topical Delivery Potential [27,28]

- Being well balanced in hydrophilic and lipophilic character, they can efficiently partition with the skin and therefore enhance the skin penetration and transport of the molecules.
- Lecithin organogels also provide the desired hydration of skin in a lipid-enriched environment so as to maintain the bioactive state of skin.
- Lecithin might influence the structure of the skin by disorganizing the lipid layer in the stratum.

Limitations of Organogels

- In the lecithin organogels, the lecithin should be pure otherwise no gelling will occur.
- Lecithin is most costly.
- Lecithin is not available on large scale.
- Should be stored in a proper condition.
- The organogel has greasy property.
- Less stable to temperature.

Organogelling Composition

The organogel matrix chiefly consists of surfactant (lecithin) as gelation molecules, a non-polar organic solvent as external or continuous phase and polar agent, usually water. Lecithin is a trivial name for 1,2-diacyl-Sn-3-phosphocholine. It belongs to a biological essential class of substance termed phosphoglycerides or phospholipids. The latter form the lipid matrix of biological membrane and also play a key role in the cellular metabolism.

As a biocompatible surfactant, it is widely used in everyday life including human and animal food, medicine, cosmetics and manifold industrial applications [29]. Synthetic lecithin containing residues of saturated fatty acids failed to form organogel [27,30]. However, it has been established that unsaturation in

phospholipid molecules is a desired property for the formation of lecithin organogels. Besides lecithin as gelation molecules, the role of organic solvent in providing the desired solvent action into the gelatin molecules is much emphasized. A large variety of organic solvent are able to form gel in the presence of lecithin. Among them are linear, branched and cyclic alkenes, ethers and esters, fatty acids and amines. Specific examples includes ethyl laurates, ethyl myristate, isopropyl myristate (IPM), isopropyl palmitate (IPP), cyclopentane, cycloclane, n-pentane, n-hexane, n-hexadecane and tripropylamine [25].

Amongst the above, the fatty acid esters i.e., application of lecithin organogels. This has been attributed to their skin penetration enhancing property besides their biocompatible and biodegradable nature [29,31].

The third component of polar agent acts as a structure forming and stabilizing agent and has a very crucial role to play in the process of gelling. Water is the most commonly employed polar agent although some other polar solvents such as glycerol, ethylene glycol and form amide have also been found to possess the capability of transferring an initial non-viscous lecithin solution into jelly like state on organogel [22].

As described earlier, the major limitation in formation of lecithin organogels is the requirement of high purity lecithin, the high purity grade lecithin is not only expensive but also difficult to obtain in large quantities. However, recent reports indicates the incorporation of synthetic polymers i.e., pluronic in lecithin organogels, for their usefulness as co-surfactant and stabilizer [32]. It has been shown that the inclusion of pluronic as cosurfactant makes the organogelling

feasible with lecithin of relatively lesser purity [33]. The term “pluronic” refers to series of non-ionic closely related block copolymers of ethylene oxide and propylene oxide [29]. These are primarily used in pharmaceutical formulations as co-surfactants, emulsifier, solubilizers, suspending agents and stabilizers. These pluronic containing lecithin organogels have been termed as pluronic lecithin organogels, poloxamer organogels, pluronic organogels, PLO gel or simply PLOs.

Method of Preparation

The oil-surfactant mixture is heated at 60°C to obtain a clear solution which on cooling forms organogels [34]. Based on the phase diagrams constructed, lecithin solutions are prepared by first dissolving lecithins in an organic solvents with the aid of magnetic stirrer. Formation of organogels takes place on addition of water with the help of micropipette syringe. Sometime heat is applied for complete solubilization of drug [26].

The oil phase is prepared by mixing lecithin and organic solvent, the mixture is allowed to stand overnight to ensure complete dissolution. The aqueous (polar) phase is prepared by adding pluronic to ice cold water, the mixture is agitated to ensure complete dissolution. The prepared PLO, the oil phase is mixed with aqueous phase of pluronic using a high shear mixing method by magnetic stirrer [32].

Characterization of Organogels

In contrast to the ease of preparation, characterization of LOs is relatively complicated on account of their interior structural design build up on the self-associated supramolecules. These microstructures, the resultant of varied polar non polar interactions, are highly sensitive and pose difficulties in the investigative studies. However, different

characterization studies are extremely useful while investigating the potential applications of organogel systems as a topical vehicle. For instance, it has been reported that many of the physicochemical properties of LOs viz. Rheological behavior, physical and mechanical stability, and drug release behavior are dependent upon how do molecules arrange themselves to provide the specific structural network within the organogel system [22, 35].

Phase-behavior of organogels

For any vehicle to be used for topical drug delivery applications, it is essential to study its rheological behavior. The latter is important for its efficacy in delivering the molecules onto or across the skin site. The critical parameters like spreadability, adhesiveness (property related to bioadhesion on skin site), cohesiveness (which indicates structural reformation following application of shear stress, and consistency need to be modified in a favorable manner. Lecithin organogels (LOs) have been studied extensively for their rheological attributes and determined to be viscoelastic in nature [22].

At higher lecithin concentrations, there is more extensive entanglement of long cylindrical micelles with each other, forming a network-like structure with a very high viscosity. The entrapment of the drug within this network lowers the amount of free drug available for release, causing a decrease in the release across the membrane [26].

Sameles containing different weight ratios (k_m) of lecithin/IPM (20:80) (40:60) (60:40) (80:20) are generally prepared, phase studies are carried out by adding water while stirring. After each addition of 1 μ liter of aqueous phase of pure water to the lecithin solutions, the resulting systems are examined for clarity and viscosity. The course of each addition is monitored

through cross polaroids in order to determine the boundaries of any organogel and birefringent liquid crystalline domains. The endpoint of the organogel domain at a given k_m is determined when the system became turbid after the addition of a specific amount of water. The phase behavior of the systems is mapped on phase diagrams with the top apex representing the lecithin and the other apices representing IPM and water solution. The transparent, homogeneous, nonbirefringent area enclosed by the line connecting the endpoints are considered as microemulsion based organogel [35].

Organogel structure and mechanism of organogelling

The initially spherical reverse micelles that are formed by lecithin molecules in a nonpolar organic solvent transform into cylindrical micelles, once water is added. This is established with the help of light scattering and small angle neutron scattering techniques. This one dimensional growth of micelles is caused by the formation of hydrogen bonds between water molecules and phosphate groups of lecithin molecules so that two adjacent lecithin molecules are bridged together by one water molecule. IR and NMR spectroscopic methods have revealed that water molecules could interact simultaneously with phosphate groups of neighboring lipid molecules via hydrogen bonding, acting as a bridge between them. In this case solvent molecules and lecithin phosphate groups can arrange in such a way that a hydrogen-bonded network will be formed. The increase in the amount of water results in the formation of long tubular and flexible micelles. These micelles can be entangled and therefore build up a transient three-dimensional network, that is responsible for the viscoelastic properties of the lecithin

organogels. At a critical concentration of water, network shrinks and phase separation occurs. At still higher contents of water a transformation to a solid, nontransparent precipitate can be observed. This diluted solution is composed of rod-like micelles, which their length is not enough to overlap and form a three-dimensional network. The existence of microdomains of different polarity within the same single-phase solution enables water-soluble and oil-soluble drugs to be incorporated. This could be attributed either to the increase in the number of cylindrical micelles or to the further growth of the cylindrical micelles or both, leading to the increase in the solubilizing capacity [35].

Determination of gelation temperature

Formulations are enclosed in glass tubes (2 mm inside diameter) and observed over a temperature range of 4-5°C. The change from solution to gel or vice-versa are determined by inverting the tube. The temperature is changed at a rate of 5°C h⁻¹ and the temperature at which the physical state of the formulation changes is regarded as the gelation temperature [36].

Gelation Kinetics

The gelation properties of organogels are investigated in the presence of various solvents. Gel-sol and sol-gel transitions were evaluated by the inverse method and gelation kinetics are determined by turbidimetry [37].

In Vitro Drug Release

The permeation apparatus designed as described by Chowdary *et al.* is employed to study the release profile of drugs from the semisolid formulations. The release/permeation of drugs from lecithin gels through various membranes is determined using Franz diffusion cell [38].

Application and Future Prospects

In the field of topical drug delivery, LOs have emerged as one of the most potential carrier systems. In contrast to other lipid-based system such as vesicular system (liposomes and niosomes) lecithin-organogel systems may prove to have an edge in term of efficacy, stability and most importantly, the technological feasibility. Moreover, the topical drug delivery of new biotech generated proteinaceous molecules in the protective non-polar microenvironment of these systems may help protect these sensitive macromolecules from and degradation, while their transport to the desired site. Thus, amidst the increasing opportunities and challenges, the LOs may prove to be highly promising system in realizing the drug delivery objectives while scientists are desperately trying for more viable alternative viz-a-viz existing carrier system. PLO is probably due to financial constrains as well as the industry focusing on area such as biotechnology and genomics. However, the great interest in PLO in the US has led to formulation of a second generation lecithin organogel premium, lecithin organogel base by Xenex Labs and Max Pharmaceuticals, USA. Table 1 shows some of the application and major findings of lecithin based organogels. A gel using hydroxy propyl cellulose and ethanol was formulated for transdermal delivery of testosterone. Testosterone loaded in the gel was 21 mg/cm² [39]. Similarly, a hydrogel formulation of fentanyl or sufentanil was prepared using polyvinyl alcohol and resin buffer. The formulated gel had a skin contact area of 2 cm² and 0.16 cm², respectively. The approximate weight of gel was 350 mg. This gel was delivered by electrortransport over 20 min in a dosage of 4 µg-5.5 µg [46]. The composition of

transdermal formulation patented by Murdock et al. (2002) is summarized in Table 2. The workers extended this study to a combination of two active ingredients for the treatment of painful spasticity. Amitriptyline appeared to offer limited pain relief when administered

transdermally. The results revealed that the combination of gabapentin with doxepine might offer additional benefit. The addition of guaifenesin to doxepine was proposed to be of particular value in cases of painful spasticity [47].

Table 1: Applications and Major Findings of Lecithin Organogel-Based Systems

Organogel formulation	Application/Major findings	References
Lecithin (200mM) IPP gel of broxaterol and scopolamine	Transdermal delivery of compounds	39
Phosphatidylcholine (PC) gel in isopropyl palmitate (IPP) or cyclooctane	Investigated for transdermal transport of various drugs along with amino acids and peptides	27
IPP-lecithin gel of diclofenac and indomethacin	Enhanced efficacy of NSAIDs administered through topical route	40
Phytosphingosine or sphingosine lecithin organogel comprising soy PC, IPP, ethanol and water	Treatment of scars	41
Soya lecithin-isopropyl myristate (IPM) organogel containing ketamine hydrochloride and amitriptyline hydrochloride	Enhance skin penetration and partitioning of the drugs into the skin layers	42
Nicardipine lecithin-IPM organogel	Enhanced skin permeation across guinea pig and human skin	43
Methimazole in LO gel	Significant percutaneous absorption of methimazole	28
LO gel of cardiac glycoside digoxin	Topical administration of the compound in LO gel was found to be effective for the treatment of muscle spasm	44
Cyclobenzaprin in lecithin organogel (Lecithin 10-30%, IPM 10-30%, water 30-60%)	Topical formulation for bauxism.	45
Ketoprofen PLO gel	Administration of ketoprofen in PLO gel offered convenience, produced less side effects and alleviated pain in a specific location	27
PLO gel of Diclofenac, Ibuprofen, Ketamine	Randomized, placebo controlled study on lateral epicondylites employing diclofenac in PLO gel reduced pain and increased functional status	45
Lecithin organgel in combination of Pluronic F-127 (poloxamer 407) solution/ Cyclobenzaprin	Effective formulation for topical treatment of carpal tunnel syndrome	33
Lecithin (20-40% v/v) in isopropyl palmitate or isopropyl myristate containing suitable amount of pluronic and water with or without short chain alcohol	The components of PLO gel provide desired hydration state to the skin, thus effective in the treatment of eczema or psoriasis	41

Table 2: Transcutaneous Absorption of Various Drugs Formulated into Lecithin-Organogel Systems [41]

Active ingredient (mg/ml)	Ingredients*	Dose/day (mg)	Time when blood was withdrawn (days)	Blood serum level (ng/ml)	Reference level (ng/ml)	Remarks
Paroxetine HCl (20)	Etoxydiglycol	40	210	0	49 + 0.26	Poor absorption/ lab error.
Sertraline (15)		100	19	5	30 + 200 mg/ml	Limited absorption/ lab error
Fluoxetine HCl (10)	Ethyl alcohol, Isopropyl myristate	20	7	45	-	Patient benefit
Carbamazepine (150)	Etoxydiglycol	400	120	4.6	4-10µg/ml	Good absorption, No GI side effects and clinical improvement
Carbamazepine (150)	Etoxydiglycol	200	60	10.8	4-10µg/ml	Excellent absorption, No GI side effects and clinical improvement
Bupropion (15)	Water	100	44	>0.5	10-30	Poor absorption, lab error, patient non compliance

* Pluronic F-127 gel and soya lecithin were present in different amounts in all formulations.

CONCLUSION

Lecithin, a biocompatible material has recently gained wide popularity for development of better drug delivery system. Interest in organogels has increased in a wide variety of fields including chemistry, biotechnology and pharmaceuticals. Lecithin based delivery systems appear to be unique and industrially feasible approach to overcome the problem of low bioavailability associated with the lipophilic drugs. Lecithin based organogels as transdermal drug delivery provides the promising delivery system for lipophilic drugs. It provides better patient compliance by

avoiding invasiveness, prolonging plasma drug level, bypass first pass effect, reduced side effects and easy termination of therapy.

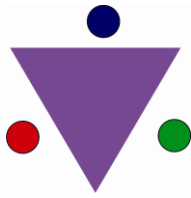
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EFFECT OF CORE STABILIZATION EXERCISE ON A TRUNK EXTENSORS ENDURANCE TRAINING PROTOCOL

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ABSTRACT

Objective: The objective of the study was to find the effectiveness of core stabilization exercises over a trunk extensor endurance training protocol (TEEP) in improving trunk extensor endurance.

Methods: 30 physiotherapy students in the age group of 18-23 from KJPCP were conveniently selected and randomly allocated to a control and experimental group having 15 subjects each. Experimental group was given a trunk extensor endurance protocol along with a core stabilization program and control group was given only TEEP. Both exercises were given for half an hour each, 6 days a week, for 6 weeks.

Results: It was found that core stabilization exercises along with TEEP is not significantly different from trunk extensor endurance training protocol alone in improving endurance of trunk extensors.

Conclusions: TEEP protocol alone is effective in improving trunk extensor muscle endurance and its combination with core stabilization exercises will not produce any significant improvements over use of TEEP alone.

Key words: Trunk extensor endurance training protocol (TEEP), core stabilization exercises, Modified Sorenson test.

INTRODUCTION

Muscular endurance is the ability of an isolated muscle group to perform repeated contraction over a period of time, with intensity of the activity being moderate.¹

It is one of basic elements of muscular performance that has great relevance to activities of daily living, lifting and bending in which the ability of trunk extensor to resist fatigue being important in industrial setting². Poor endurance of trunk muscle may induce strain on passive structure of lumbar spine and hence result in low

back pain^[1,7]. Muscle has already been identified as a potential source of low back pain^[4,5] as failure to protect passive structure from excessive loads may result in damage to pain sensitive structure and produce pain^[6,7,12].

Endurance of lumbar stabilizer is a very important key for preventing lumbar pain^[8,9,10]. Trunk muscle endurance training has been recommended as means of increasing fatigue threshold and improving performance and reducing disability.^[11]

Improving endurance of trunk extensor therefore appears to be sound and promising approach for preventing low back pain and hence justification for conducting this study among individual without low back pain^[12,20,21].

The trunk extensor training protocols used in previous studies focused extensively on erector spinae composed of longissimus and spinalis, that is on a mobilizer of trunk at expense of stabilizers such as transverse abdominis and multifidus that are affected majorly in individuals with back pain^[13,22].

Even though core stabilization exercises are being used widely in clinical setting its effect on dynamic stabilizers of spine remains unexplored. Trunk extensor endurance training protocol may need to be used in conjugation with specific stabilizing exercise of multifidus and transverse abdominals for a better result in back problems in patients. Core stabilization exercise through effective abdominal training is supposed to increase ones strength and stamina. So the training of trunk/ spinal stabilizers is therefore supposed to help in improving the endurance of trunk extensors or mobilizers and preventing potential development of backache in the future. It would of great importance if core stabilization programs with the emphasis on abdominals are found to have an effect on extensors of spine. Thus the purpose of this study is to investigate whether the application of core stabilization exercise along with a trunk extensor endurance protocol can improve trunk extensor endurance than the use of extensor training protocol alone in apparently healthy subjects.

MATERIALS AND METHODOLOGY

Materials

1. Rectangular Wooden Box (80x50x20.3cm)
2. Stop Watch
3. Weighing scale
4. Height meter/inch tape
5. Velcro Straps
6. Two pillows
7. Data collection sheet and pen

Methodology

30 normal healthy subjects from the campus of K.J.P College of physiotherapy within the age group of 18-23years were recruited for the study, on the fulfillment of inclusion criteria. A two group pre-test-post-test non randomized controlled trial design was used for subjects being recruited through convenience sampling. The study procedure and rationale were explained to subjects and their informed consent of participation obtained. Subjects were conveniently recruited but randomly assigned into either a controlled or experimental group by asking them to pick a piece of paper on which either E (experimental) or C (control) was inscribed. Age as on last birthday and gender were noted while their body weight and height were measured and recorded using standardized procedures. Their Body Mass Index was then estimated as weight in Kilo grams divided by height in meters square.

The subjects in experimental (B) group were given a standard trunk extensor endurance training protocol (TEEP) previously used by Babatunde O.A et al (2007)¹⁴, 4 days a week along with core stabilization exercises for the same number of days. Five standard core stabilization exercises, with the emphasis on abdominal activation, as given by 'Therapeutic exercises', 5th ed were used. All the exercises were started in supine hook lying position. First exercise was abdominal activation using 'drawing in' procedure as in standard core stabilization methods. Second exercise was hip knee flexion extension of alternate legs up to 90 degrees of knee flexion, dragging the feel on bed. Third exercise included flexion extension of alternate legs, without dragging, up to 90 degrees of hip knee flexion. Fourth exercise was flexion extension of both legs together up to 90 degrees of knee and hip flexion without dragging of foot. Fifth exercise was straight elevation of lower limbs up to 45 degrees. In

each exercises abdominals were contracted for 10 seconds. Each exercise was repeated 25 times per session in subject's comfortable speed and those who required for break were given a single break of 30 seconds during each exercise. In between different exercises each subjects were given 1 minute rest. Duration of each exercise was between 25-30 minutes per day. The control (A) group had under gone only TEEP for the same duration and number of days. The outcome measure was the trunk extensor muscle endurance (TEME) using modified Sorenson test. TEME of both groups were calculated on day 1 and after 6th week. And the 6th week score of both E and C groups were also compared.

Statistical methods

Statistical test used was student's t Test. With in groups Pre and post test scores were compared using paired t test and In between group pre and post test group scores were compared using unpaired t test. Level of significance was kept at 0.05. Data was analyzed using SPSS version 14.

RESULTS

Pre test scores didn't show significant difference between two groups proving the homogeneity of the groups..Group E did show significant difference between pre and post test scores($t=5.043$ sig=.000).Similarly group C also did show significant difference between pre and post test scores($t=5.386$,sig=.000).But on comparison of post test scores between two groups, it was found that there were no significant differences($t= -1.499$,sig=.145).

DISCUSSION

From the analysis it was seen that there was significant difference between pre test- post test scores of both groups but no statistically significant results were found between the post

test scores between the group A (TEEP) and group B(TEEP + core stabilization) .

The reason for insignificant difference would be that core stabilization exercises might not be so much effective in improving trunk extensor endurance of normal population either because of the inability of normal subjects in proper activation of core stabilizers or the duration of study might not be so much effective in getting results in normal healthy population. The study was conducted for four days weekly for one and a half month taking less than half hour per day, so there may be need to increase the duration of the study to get a statistically significant outcome in normal healthy population over a trunk extensor training protocol.

In the study conducted by Cairns, Mindy C.; Foster, Nadine E.; Wright, Chris on September 2006 it was concluded that there was no effect of core stabilization exercises on recurrent low back pain^[15,23]. That study used 2 groups: conventional, physiotherapy consisting of general active exercise and manual therapy; and conventional physiotherapy plus specific spinal stabilization exercises. Both groups showed improved physical functioning. No statistically significant differences between the 2 groups were shown for any of the outcomes measured, at any time and there was no additional benefit of adding specific spinal stabilization exercises to a conventional physiotherapy package for patients with recurrent LBP. Similarly when studies were conducted on healthy subjects it was concluded that there was no effect of core stabilization on trunk extensors. George A Koumantakis, Paul J Watson and Jacqueline A Oldham on March 2005 examined the usefulness of the addition of specific stabilization exercises to a general back and abdominal muscle exercise approach for patients with sub acute or chronic nonspecific back pain by comparing a specific muscle stabilization-enhanced general exercise

approach with a general exercise. But there were generally no differences between the 2 exercise approaches for any of their outcomes other than self reported disability¹⁶.

They concluded that a general exercise program reduced disability in the short term to a greater extent than a stabilization-enhanced exercise approach in patients with recurrent nonspecific low back pain. The mode of action of stabilization retraining still remains unclear, because it has not been shown to be capable of mechanically containing an unstable segment, even upon improvement of muscle activation. Stabilization exercises do not appear to provide additional benefit to patients with subacute or chronic low back pain who have no clinical signs suggesting the presence of spinal instability. Similarly in healthy subjects correct contraction of the stabilizing muscles could not be achieved initially and subjects had to be constantly corrected by the treating physical therapist also each time new exercises were introduced^[17,18]. This too can be attributed to the minimal effect of core stabilization exercises on the experimental group. Professor Eyal Lederman in his article 'myths of core stabilization' concluded that Core stability exercises are no more effective than, and will not prevent injury more than, any other forms of exercise. Core stability exercises are no better than other forms of exercise for back care¹⁹.

In the light of existing literature it can be easily concluded that core stabilization exercises have controversial validity and their inability to produce significant changes to trunk extensor endurance in normal healthy population in comparison to a specific trunk extensor endurance training protocol is well in sync with their controversial validity.

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Table 1 : Mean and Standard deviation of group A (week 0-week 6)

Week	Mean	N	Std Deviation	Std Error mean
ZERO	52.7720	15	22.7747	5.8804
SIXTH	76.6120	15	27.6709	7.1446

Table 2 : Within group comparison of group A(week 0-week 6) using *paired t test*

	Paired Differences					t	df	Sig (2 tailed)
	Mean	Std Deviation	Std Error mean	95% Confidence interval of the difference				
				Lower	Upper			
Week 0-week 6	23.8400	17.1437	4.4265	14.3461	33.3339	5.386	14	.000

Table 3: Mean and standard deviation of Group B (week 0-week 6)

Week	Mean	N	Std Deviation	Std Error Mean
Zero	61.2073	15	37.7894	9.7572
SIXTH	97.7207	15	32.5235	8.3975

Table 4: Within group comparison of group B (Week 0-week 6) using *paired t test*

Pair	Paired differences					t	df	Sig (2 tailed)
	Mean	Std Deviation	Std Error mean	95% confidence interval of the difference				
				Lower	Upper			
Week -0 Week-6	36.5133	28.0410	7,2403	20.848	52.0419	5.043	14	.000

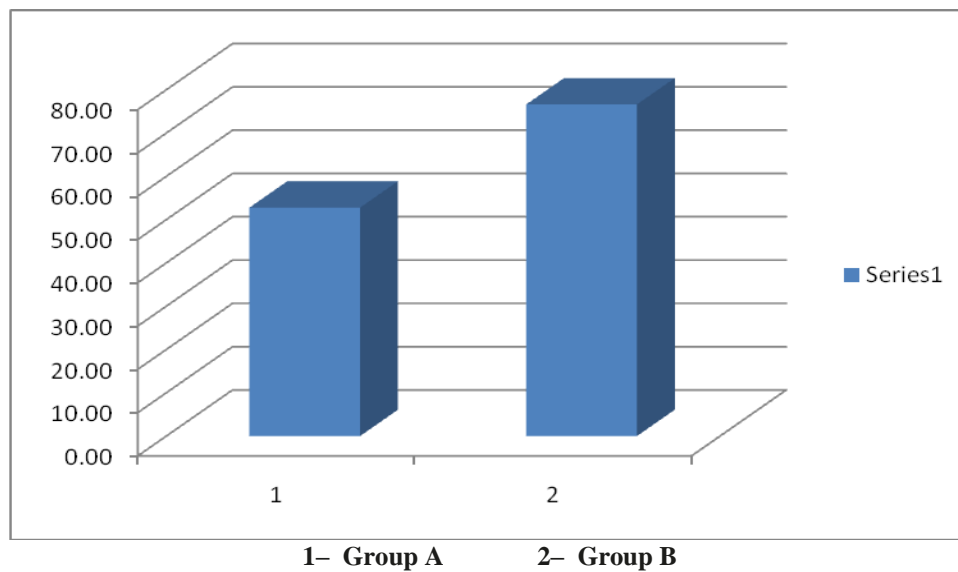
Table 5 . Comparison of mean and standard deviation between group A and group B.

Groups	Numbers	Mean	Std Deviation	Std Error mean
Group A	15	23.7960	17.1188	4.4202
Group B	15	36.5133	28.0410	7.2402

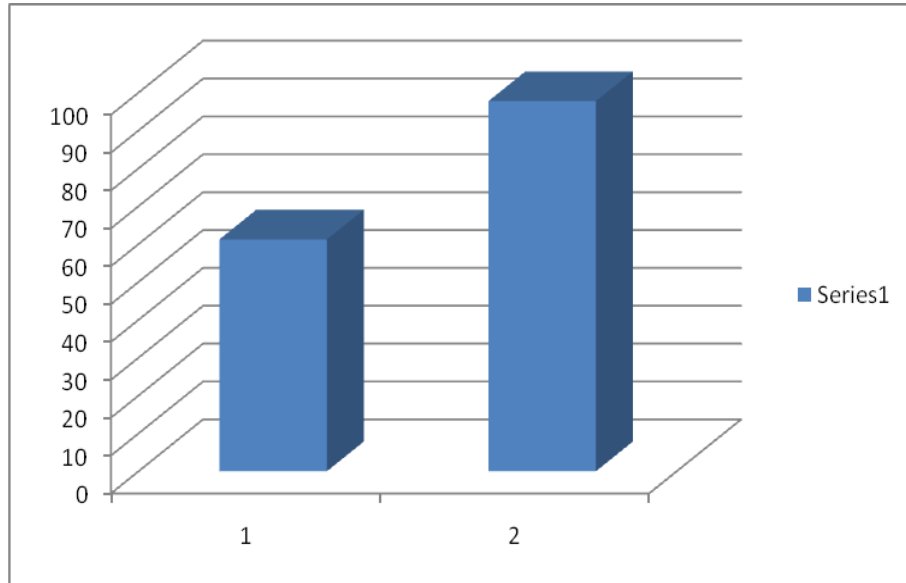
Table 6. Analysis of post-test scores of Group A and Group B using *unpaired t test*

	Levenes test		t-test for equality of means						
	F	Sig	t	df	Sig(2 tailed)	Mean difference	Std Error Difference	95% confidence interval of difference	
								Lower	Upper
Equal variances assumed	2.481	.126	-1.499	28	.145	-12.713	8.4827	-30.093	4.658
			-1.499	23.163	.147	-12.7173	8.4827	-30.258	4.823
Equal variances Not assumed									

Graph 1 : Group A : Comparison of mean values of pre and post test scores

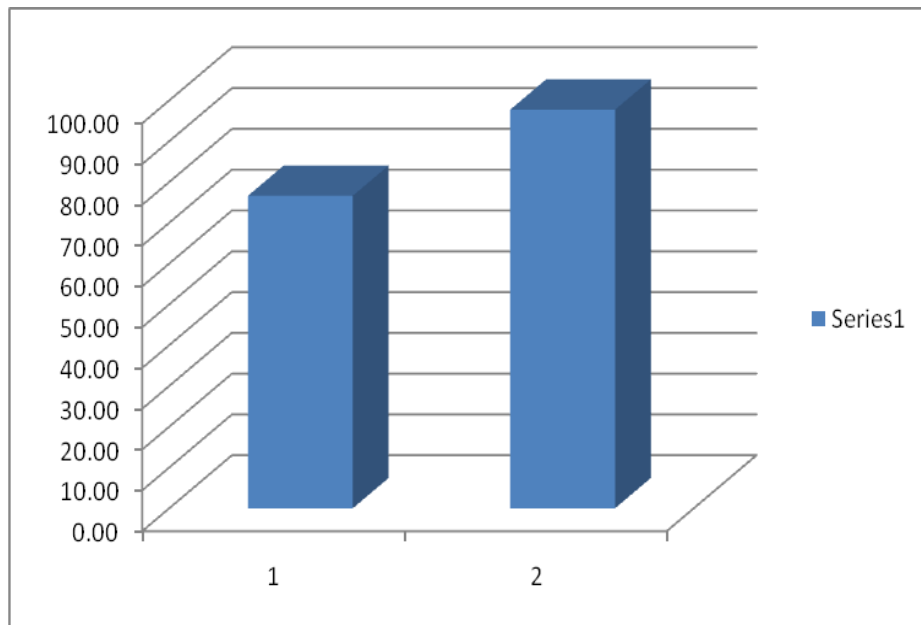


Graph 2: Group B: Comparison of mean values of pre and post test scores

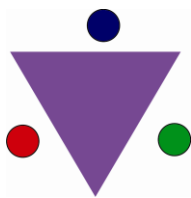


1- Group A 2- Group B

Graph 3 Comparison of mean values of post test scores of Group A and Group B



1- Group A 2- Group B



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LIPOPROTEIN LIPASE GENE POLYMORPHISM AT *Pvu II* RESTRICTION SITE AND CORONARY ARTERY DISEASE

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ABSTRACT

The present study was made to find the *PvuII* restriction enzyme polymorphism of lipoprotein lipase (LPL) gene and its association with angiographically defined coronary artery disease (CAD). Lipoprotein lipase (LPL) plays a central role in lipid metabolism by hydrolyzing triglyceride in chylomicrons and VLDL. Polymorphic variants of the LPL gene are common and might affect risk of CAD. Two milliliter of blood samples were collected from 30 patients, and 30 control subjects. Genomic DNA was isolated from the blood samples and subjected to PCR-RFLP analysis for determination of the genotype with regard to the *Pvu II* polymorphism of LPL gene. The amplified product was digested with restriction enzyme *Pvu II* and genotyped by through electrophoresis in 2.5% agarose gel. For the *PvuII* genotypes, within the CAD group(n=30), the +/- genotype (+ presence of *Pvu II* restriction site, - absence of *Pvu II* restriction site) was found in 12 individuals (40%), whereas 10(33%) carried the +/+, and 8(27%) carried the -/- genotype. In the control group (n=30), the -/- genotype was found in 6 subjects (20%), 13 (43%) carried the +/+ genotype and 11(37%) carried the +/- genotype. The genotype frequency distribution was significantly different in the CAD and control study group. The most frequent genotype among CAD patients was (-/-), this genotype was more frequent in patients than in control subjects. There was a difference in the distribution of LPL-*PvuII* genotypes between the healthy subjects and the patients with CAD. The common LPL polymorphic allele, the *PvuII* (+) or (-) is modestly associated with CAD. Genetic variants of LPL deserve further evaluation as risk factors for CAD.

Keywords: Lipoprotein lipase; coronary artery disease ; *pvu II* restriction site ; Enzyme

INTRODUCTION

Coronary Artery Disease (CAD) is the most common form of heart disease. This condition occurs when the coronary arteries, that supply oxygen- rich blood to the heart muscle, gradually become narrowed or blocked by plaque deposits. The plaque

deposits decrease the space through which blood can flow. Poor blood flow can 'starve' the heart muscle and lead to chest pain. A heart attack results when blood flow is completely blocked, usually by a blood clot forming over a plaque that has ruptured (Lusis *et al.*, 2000).

Lipoprotein lipase (LPL; triacylglycerol-protein acyl hydrolase, EC 3.1.1.34) catalyzes the hydrolysis of core

triacylglycerol of circulating very low density lipoprotein(VLDL) and chylomicrons generating fatty acids for storage in adipose tissue or oxidation in muscle. This enzyme therefore plays a crucial role in plasma lipoprotein processing and energy metabolism in general (Eckel,1987).LPL is a glycoprotein synthesized and secreted by a variety of parenchymal cells, including adipocytes,skeletal and cardiac muscle cells and macrophages. After secretion it becomes bound to glycosaminoglycans on the luminal surface of capillaries. Apolipoprotein CII, which is present on the surface of VLDL and chylomicrons, was shown to act as a cofactor for LPL (Olivecrona *et al.*, 1997).

The importance of LPL in the regulation of overall lipid metabolism and transport in humans and other animals is well documented (Cryer, 1981).LPL is expressed by the parenchymal cells of extra hepatic tissue and catalyses the hydrolysis of the triacylglycerol component of chylomicrons and very low density lipoprotein (VLDL),thereby providing non-esterified fatty acids and 2-monoacylglycerol for tissue utilization .Because the circulating lipoproteins are in general, too large to cross the vascular endothelial cells, to which the enzyme is attached via highly charged membrane bound chains of heparin sulfate proteoglycans (HSPG) (Godota *et al*, 1992) .Although adipose tissue and muscle parenchymal cells are the major source of LPL synthesis, from where the mature enzyme is secreted by macrophages. It is the source of LPL that has been implicated to play a major role in the pathogenesis of atherosclerosis.

LPL gene, mapped to the short arm human chromosome8, contain 10 exons, separated by 9 introns (Deep *et al.*, 1989).Gene analysis showed many mutations involving the noncoding and coding sequences of the human LPL gene (Murthy *et al.*, 1996).Several restriction fragment length polymorphisms (RFLPs) have been identified at the LPL gene. The occurrence of alternate types of nucleotides at the same position in the nucleic acid sequence with no concomitant apparent phenotypic differences is generally referred to as polymorphism and it can be easily detected if it leads to restriction site alterations. The most extensively investigated of these are the *PvuII* (Fisher *et al.*, 1987) and *HindIII* (Heinzmann *et al.*, 1987) sites. *PvuII* polymorphism is the result of change in the restriction site of the LPL gene 6th intron, 1.57kb from SA site (Oka *et al.*, 1989). The region containing *PvuII* site resembles the splicing in its homology to the consensus sequence required for 3'-splicing and the formation of the lariat structure, suggesting that C497-T(CAG CTG-TAG CTG) change may interfere with correct splicing of mRNA. DNA polymorphism is a useful marker to analyze disorders with genetic backgrounds, even when the genetic cause of the disease has not been elucidated (Godota *et al.*,1992).A number of DNA polymorphism have been investigated for their possible linkage with hereditary predisposition to common polygenic disorders, such as dyslipidemia (Lusis *et al.*,1988).Several trails explored association between LPL gene for an association between genotypes identified by the *PvuII* restriction fragment length polymorphism and plasma triglyceride levels (Chamberlain *et al.*,1989;Wang *et al.*,1996),but other failed

to find significant association (**Heizmann et al.,1991;Jemaa et al.,1995**).

MATERIALS AND METHODS

SAMPLE COLLECTION

The blood samples were collected from the patients between the age group of 30-60 from inpatients and outpatient department of **Frontier Life Line, Dr.KM.Cherian Heart Foundation, Chennai**. Plasma was isolated for subsequent analysis of measuring sugar level, HDL, LDL, Total cholesterol and Triglycerides were carried out in the clinical Biochemistry laboratory of the hospital.

DNA ISOLATION

The genomic DNA was isolated from venous blood by using Phenol chloroform isolation method. The blood samples were obtained in a EDTA (Ethylene diamine tetra acetic acid) coated vacutaines. From this 1ml of the blood was taken into an eppendroff tube and centrifuged at 3000rpm for 30minutes. On centrifugation a white Buffy coat (which has the WBC) was obtained which is removed and transferred to fresh eppendroff tubes. To this, thrice the volume of lysis buffer (0.155M ammonium chloride, 0.1M potassium bicarbonate and 0.1mM EDTA) was added; then Mixed and stored at -20°C for 30minutes which would allow cell lysis to occur. Later the tubes were centrifuged at 2000 rpm for 5 minutes and the supernatant was discarded. To the pellet, 200µl of 0.5M sodium EDTA, 100µl of 10% SDS and 10µl of proteinase k (100µg/ml) were added and left for overnight incubation. The next day to this overnight solution, equal volumes of Tetrahydroxy aminomethyl hydrochloric acid (Tris) saturated phenol (pH 8) was added and mixed well in an overhead shaker for 30minutes. The tubes were centrifuged at

6000 rpm for 10minutes and the upper aqueous layer was removed without disturbing the lower organic layer (containing phenol). To the aqueous layer again obtained equal volumes of Tris saturated phenol, Chloroform and isoamyl alcohol in the ratio 25:24:1 were added and mixed in an overhead shaker for 30minutes. Again the upper aqueous supernatant was taken and further residual phenol was removed by extracting with equal volumes of chloroform and isoamyl alcohol (24:1) and mixed well in an overhead shaker. To the aqueous layer, one tenth the volumes of 3M sodium acetate and equal volumes of chilled ethanol was added and shaken well. The tubes were centrifuged at 12000 rpm for 2 minutes and the supernatant was discarded. The pellet was washed with 70 % ethanol and the DNA obtained was stored in TE (1M Tris, 1mM EDTA) at 4°C.

POLYMERASE CHAIN REACTION

Polymerase chain reaction (PCR) is a technique which is designed to permit selective amplification of a specific target DNA sequence or sequences within a heterogeneous collection of DNA sequences. The *PvuII* genotypes were determined by polymerase chain reaction (PCR) amplification of the polymorphic regions found in intron 6 region of the lipoprotein lipase (Oka *et al.*,1989), followed by digestion of these amplified fragments with *PvuII* restriction endonuclease. The *PvuII*-containing site was amplified using the following primers (Sigma, Bangalore).

Forward 5'- CATCCATTTTCTTCCACAGGG -3'
Reverse 5'-TAGCCCAGAATGCTCACCAGACT- 3'

PCR reaction amplification was carried out in 15 µl reaction volume containing, 1X PCR buffer, 1.5mM MgCl₂, 200µM dNTPs, forward and reverse primers 0.5µM respectively, 0.5U Taq DNA polymerase (Sigma, USA) and Genomic DNA (50ng – 100ng). The thermo cycling procedure was carried out using Thermocycler (MJ Research Inc, USA) for 30 cycles. PCR cycling conditions were 94°C for 5 minutes for initial denaturation followed by 30 cycles of 94 °C denaturation for 45 seconds, 54°C annealing for 30 seconds, 72°C extension for 30 seconds, 72 °C final extension for 3 minutes.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

The amplified DNA sample was subjected to restriction digestion with 5 units of *Pvu II* restriction enzyme (Biogene.U.S.A). It was incubated at 37 °C for 3 hours. Then the digested sample was subjected to 2.5% agarose gel electrophoresis.

GEL ELECTROPHORESIS

The gel template was cleaned and its edges were sealed. A solution of 2.5% agarose gel (1X Tris borate EDTA) was prepared and the solution was warmed until the Agarose melted. The melted gel was cooled and 3µl of ethidium bromide was added (10 mg/ml stock). The warm solution was poured into the template and allowed to solidify. The comb and the seal were carefully removed from the solidified gel. The gel was placed in electrophoresis tank and it was filled with 1X TBE buffer. The DNA sample was mixed with loading dye. The sample was loaded carefully with the help of micropipette. The electrode was connected and a voltage of 1-5 volts per cm was

applied. The current supply was stopped when the dye had migrated above 70%. The gel was placed carefully on an ultraviolet transilluminator and the DNA bands were viewed. The gel was then photographed and developed.

STATISTICAL ANALYSIS

The mean, standard deviation were calculated for each parameter (age, BMI, glucose level, etc.) for both the control and patient samples.

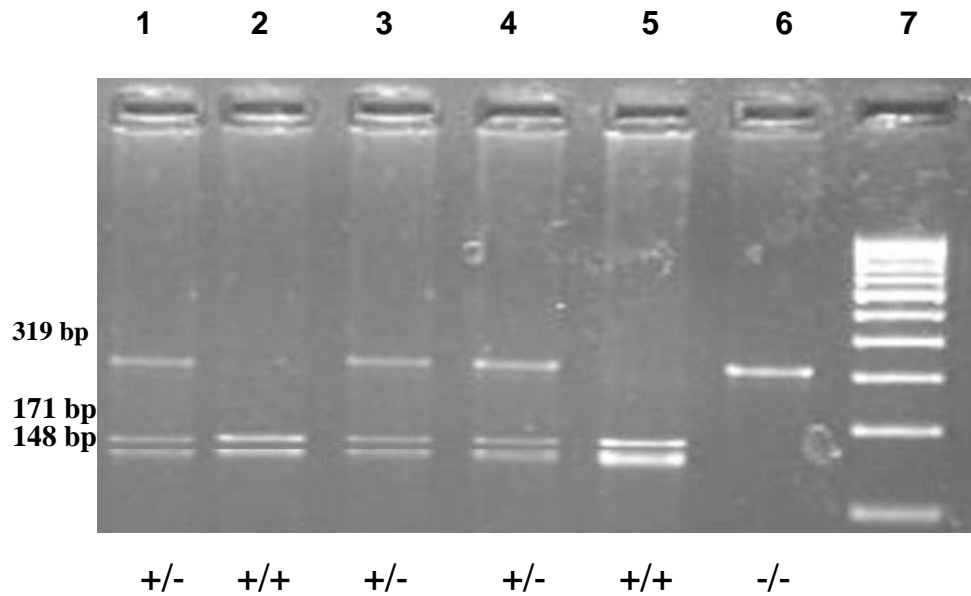
The standard deviation of a random variable X was defined as:

$$\sigma = \sqrt{E((X - E(X))^2)} = \sqrt{E(X^2) - (E(X))^2}$$

RESULT AND DISCUSSION

The baseline features and biochemical parameters of both control and patients were listed and expressed as mean ± Standard Deviation in the **Table-1**. The number of genotypes of *Pvu II* restriction site of LPL gene was computed by gene counting method from the documented gels (Figure 1). The mean age of patients and control subjects was 47.3 ± 11.62 and 59.9 ± 8.57 years respectively. Among 30 involved patients 25 were male and 5 were female. The patients group presented convincingly lower mean levels of body mass index than control subjects. The mean value of low density lipoprotein level and high density lipoprotein level in patients were lower than the control.

Figure 1: The documented gel illustrating the PCR – RFLP *Pvu* II Polymorphism



Lane 7 – 100bp DNA ladder

Lane 2 & 5 – Homozygote Presence of *Pvu*II Restriction site

Lane 6 – Homozygote Absence of *Pvu*II Restriction site

Lane 1, 3 & 4 – Heterozygotes

Table 1: The Baseline characteristics in patients and Control subjects

Variable	Controls (N=30)	Patients (N= 30)
Age (yrs)	47.3 ± 11.62	59.9 ± 18.57
Body mass index (kg/m ²)	26.7 ± 4.9	24.7 ± 3.61
Total cholesterol (mg /dl)	194.6 ± 32.52	169.6 ± 37.25
Triglycerides (mg/dl)	173 ± 62.31	148. ± 65.4
High density lipoprotein (mg/dl)	60.2 ± 35.91	44.7 ± 21.33
Low density lipoprotein (mg/dl)	101.3 ± 46.07	96.5 ± 31.78
Glucose (mg/dl)	103.2 ± 15.49	341.5 ± 169

• **Values are mean ± SD**

The genotype and allele frequency of the *Pvu II* restriction site at the LPL locus in patients and control groups were shown in **Table-2**. The -/- homozygote among the patients and control are 27 and 20% and the +/+ homozygote among the patients and control are 33% and 43 % respectively. Also, the percent of heterozygote +/- varied significantly between the two groups 40% in

patients and 37 % in control. The +/- and -/- allele frequency were considerably higher in patients with coronary atherosclerosis than the normal control. The Chi square value in patients and controls were 1.158 and 1.511 respectively, and tested to be present in the Hardy-Weinberg equilibrium.

Table 2: LPL – PvuII Genotype and allele frequency for control and CAD subjects

Subjects	Genotype			Allele frequency		χ^2
	+/+	+/-	-/-	P (+)	P(-)	
CAD (n=30)	10 (33%)	12 (40%)	8 (27%)	0.533	0.467	1.158
Control (n=30)	13 (43%)	11 (37 %)	6 (20%)	0.617	0.383	1.511

“+” Presence of *PvuII* restriction site

“-” Absence of *PvuII* restriction site

The Chi Square and the odds ratio values were computed to prove the significance of the genotypes of patients in reference to the control. The genotypes (-/-)/(+/-), (-/-)/(+/+), (+-)/(+/+), (-/-)/(+/-) + (+/+) and (+/-) were taken for the odds ratio comparison (**Table 3**). All the odds ratio values were higher than the threshold value of one. The odds ratio was highest for the

genotype (-/-)/(+/+) (7.73 with a χ^2 value of 0.65). This was followed by (-/-) + (+/-) / (+/+) and (-/-) / (+/-) + (+/+) genotypes having an odds ratio of 1.53 (χ^2 is 0.635), and 1.45 (χ^2 is 0.373) respectively. The (-/-)/(+/-) genotype comparison had an odds ratio of 1.22 (χ^2 is 0.087). It should be noted that the χ^2 value is taken to evaluate the significance of the genotype.

Table 3: PvuII Genotype and allele frequency for control and CAD subjects

Genotype/allele	N	X ² (Chi Square)	P Value	Odds ratio	95% confidential interval
(-/-) (+/-)	30	0.087	0.768	1.22	4.658-0.321
(-/-) (+/+)		0.65	0.4201	1.73	6.631-0.453
(+/-) (+/+)		0.348	0.5552	1.42	4.531-0.444
(-/-) & (+/-) (+/+)		0.635	0.4255	1.53	4.361-0.536
(-/-) & (+/-) & (+/+)		0.373	0.5414	1.45	4.860-0.435
(+/-)		0.853	0.3557	1.41	2.911-0.681

The statistical reports for CAD and control subjects were shown in **table 4 and 5**. In control and CAD subjects, there were no significant differences in total cholesterol, HDL and TG level in common genotypes in *Pvu II* RFLPs. The total cholesterol, TG and

HDL levels were found to be highest in the +/- genotype in patients and +/+ genotype in control. The LDL level was found to be higher in the +/- genotype, both in patients and control.

Table 4: Baseline parameters Vs genotype of patients with CAD

Variables	+/+		+/-		-/-	
	Mean	SD	Mean	SD	Mean	SD
Age	61.2	8.18	59.7	9.26	58.3	9.13
Weight(kg)	64.7	12.83	66.6	13.71	62.3	9.13
Height(cm)	160.9	8.24	159.6	10.32	161.8	10.05
BMI	24.9	3.92	25	4.15	23.8	2.46
TCL(mg/dl)	160	29.85	192.1	51.21	158.6	14.3
TGL(mg/dl)	125.9	45.82	183.2	86.48	141.5	52.74
TDL(mg/dl)	94.6	30.09	102.7	46.08	92.4	10.76
HDL(mg/dl)	42.3	18.3	53.6	30.42	38	7.62
Sugar (mg/dl)	0	0	0	0	341.5	169

Table 5: Baseline parameters Vs genotype of patients with control

Variables	+/+		+/-		-/-	
	Mean	SD	Mean	SD	Mean	SD
Age	43.8	13.23	49.4	11.21	49.3	9.83
Weight(kg)	74.2	18.03	70.5	11.83	71.3	7.56
Height(cm)	166.1	9.75	162.5	10.99	165.7	4.32
BMI	27	6.99	26.8	4.05	25.9	1.79
TCL(mg/dl)	204.5	33	203.3	31.21	163.5	8.17
TGL(mg/dl)	183.8	62.42	179.5	68.9	143.2	47.81
TDL(mg/dl)	91.1	59.88	113.3	44.73	96.3	5.09
HDL(mg/dl)	80.9	45.1	53.3	27.3	38.5	5.32
Sugar F(mg/dl)	0	0	169	0	92	0

DISCUSSION

Lipoprotein lipase is a candidate gene for CAD risk, and the possible association of the LPL – *Pvu II* polymorphism to CAD was studied. The genotype distribution of LPL polymorphism at a *Pvu II* polymorphic site was investigated. The results of various association studies of LPL – *Pvu II* polymorphisms with CAD have been inconsistent.

Some previous studies have defined, an association between the extent of CAD and the LPL – *Pvu II* (+/+) genotype and the LPL – *Pvu II* (-/-) genotype to be moderately associated with CAD. Other studies did not define any significant difference in the distribution of LPL – *Pvu II* polymorphism between the healthy group and the CAD groups suggesting lack of association between any of the LPL *Pvu II* genotypes and CAD. The relevance of the *Pvu II* genotypes may vary among different populations. In the present study, the distribution of the LPL – *Pvu II* genotypes was significantly different in CAD and control study groups; the frequency of the +/- and -/- genotypes were higher in patients than in controls.

Analysis of intra genotype variance of mean values of lipid levels showed that variability of *Pvu II* in LPL contributes to a certain extent to the level and variability in serum total cholesterol and LDL cholesterol levels. The LPL *Pvu II* genotypes, the CAD subjects heterozygous (+/-) for the higher total cholesterol, triglycerides, HDL and LDL cholesterol compared with homozygous +/+ and -/- subjects.

The findings indicates no significant differences between the prevalence rates for the LPL – *Pvu II* genotypes in both the study groups, suggesting a very modest

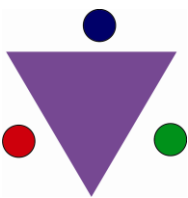
association between any of the LPL – *Pvu II* genotypes and CAD .

This study was cross – sectional raising the possibility of changes in prevalence of LPL polymorphisms among cases and control subjects due to differential survival rates for CAD patients presenting for angiography based on LPL genotype status. This seems unlikely, but can be addressed by prospective studies. Disease and control groups differed in some baseline variables, as expected. However, when differences were accounted for by conditional multivariate logistic regression, the relative risk associated with the polymorphism was maintained or augmented.

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DEVELOPMENT OF FINGERPRINTS FOR SINGLE COMPONENT ANALYSIS OF AN AYURVEDIC FORMULATION (*PIPLI CHURNA*) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Pippli Churna (PC) is a most popular Ayurvedic formulations among the Ayurvedic medicines, Piperine is one of the major constituent of Pippli Churna. The process of development of HPLC fingerprints for Pippli Churna extract is introduced in detail. The three laboratory batches and three marketed batches were taken in this study to estimate the % of piperine in this indigenous formulation. The selection of a suitable chromatographic system, the screening for important parameters, and gradient optimization to method validation, and an integrated and universal HPLC fingerprint approach was performed. This improves the separation quality of the fingerprint. The detection wavelength of piperine was 343 nm. The three laboratory batches and three marketed batches were taken in this study to estimate the % of piperine in this indigenous formulation. The results of the method validation, based on the relative standard deviation of relative retention times and relative peak areas, were acceptable. Calibration curves showed good linear regression ($R^2 > 0.999$) within test range. The LODs and the LOQs for the piperine were 0.063 mg/ml and 0.071 mg/ml. This strategy is used for the estimation of piperine in Pippli Churna(PC) formulation and identifies and assesses its quality. Results of statistical analysis show present HPLC method for determination of Piperine is simple, precise, accurate and suitable for routine analysis of Piperine in PC. The developed fingerprints can be used as a standard and Piperine can be used as a possible marker compound for fingerprinting of PC.

Keywords: Fingerprints, Pippli churna (PC), Piper species, Piperine marker, HPLC.

INTRODUCTION

India has a vast heritage of traditional system of medicine (Ayurveda, Siddha and Unani), due to the lack of precise quality control measures and finger printing methods, the benefits of these systems remains largely underutilized¹. Keeping this

thing in mind, from the last two decades, efforts have been made in developing quality control parameters for Ayurvedic formulations by means of chromatographic finger printing methods^{2,12}. World Health Organization (WHO) has emphasized the need to ensure the quality of medicinal plant products by using modern controlled technique and applying suitable standards⁸. For standardization of natural product drugs,

single chemical entities, “marker compounds,” may be used as potency standards in high performance liquid chromatography (HPLC) analysis. These marker compounds may be used to help identify herbal materials, set specifications for raw materials, HPLC analysis for marker compounds may provide additional information in the form of chromatographic fingerprints. The present study is undertaken to develop certain fingerprints for an Ayurvedic formulation. Pippli churna used for cough and cold, spleen disorders, fever, diabetes, piles, tuberculosis, abdominal disease, thrust worms, leprosy, pain (colic-spasmodic) and digestive impairments. Pippli churna possesses bioavailability enhancing properties, i.e. antitubercular drugs & antilaprotic drug. The essential oils of pipali churna have antibacterial and antifungal activities.

Ayurvedic formulary of India has given the specification for the composition of PC, it should contain piper species as a major ingredient apart from different herbs^{3,4}.

AIMS AND OBJECTIVES

Pharmacopoeial standards for Ayurvedic formulations published by the Central Council for Research in Ayurveda and Siddha gives certain physical parameters as standards for churna, these standards are not based on modern analytical methods⁵. It is therefore essential to develop definite and accurate analytical tools to as certain consistency and quality of Ayurvedic preparation from batch to batch in pharmaceuticals which may results in acceptability world wide. In present study we tried to develop a method that serves as fingerprinting method for Pippli churna(PC).

EXPERIMENTAL

All the solvents for HPLC analysis were HPLC grade and purchased from E. Merck and S. D. Fine Chemicals, Mumbai. All solvents used for extraction were primarily distilled before use. SHIMADZU – LC10AT HPLC was used for piperine analysis. All the results are obtained by repetition of the each experiment six times (n= 6).

Procurement of drug

Crude drugs were procured from local market and identified by macroscopic^{10,11} and microscopic characters^{6,7,8,9}.

Preparation of formulations

1. Three batches were prepared in laboratory (named as PC-I, PC-II and PC-III) according to strict methods of ‘Ayurvedic formulary of India’ and Sarangadhara-samhita.

2. Commercially available brands PC-A, PC-B, and PC-C, of Pippli churna were procured from local market.

Sample preparation for estimation of Piperine content

1.5 gm Pippli churna was refluxed for 1 hour with 100 ml of methanol. The volume were reduced under pressure and filtered by 0.2 µm membrane filters. The filtrate was diluted up to 100 ml with methanol. To the 20 ml of resulting solution, 2 ml of 0.5-mg/ml solution of p-dimethyl amino benzaldehyde (internal standard) was added, and made the final volume 25 ml with methanol^{7,12} [Fig. 1].

Preparation of standard solution

Piperine was purchased from Lancaster, England. Standard solution was prepared by the addition of 2 ml of solution a (1mg/ml) of Piperine and 2 ml of internal standard solution (0.5 mg/ml of p-dimethylamino benzaldehyde) in a 25 ml volumetric flask made the final volume to 25 ml with methanol.

HPLC studies⁷

Estimation of Piperine was carried from different batches (three marketed and laboratory batch) of PC with following conditions:

Column : C 18 (25cm X 4.6 mm i.d.) 10 μ ,

Mobile phase: methanol: water (69:31),

Detection: at 343 nm (reference wavelength: 343 nm),

Injection volume: 20 μ l and

Flow rate: 1.5ml/min.

Calibration

The Piperine content of PC was determined using a calibration curve established with seven dilutions, at concentrations ranging from 0.5-20 μ g/ml. Each concentration was measured in triplicate. The corresponding peak areas were plotted against the concentration of the Piperine injected. Peak identification was achieved by comparison of both the retention time and UV absorption spectrum with those obtained for standards.

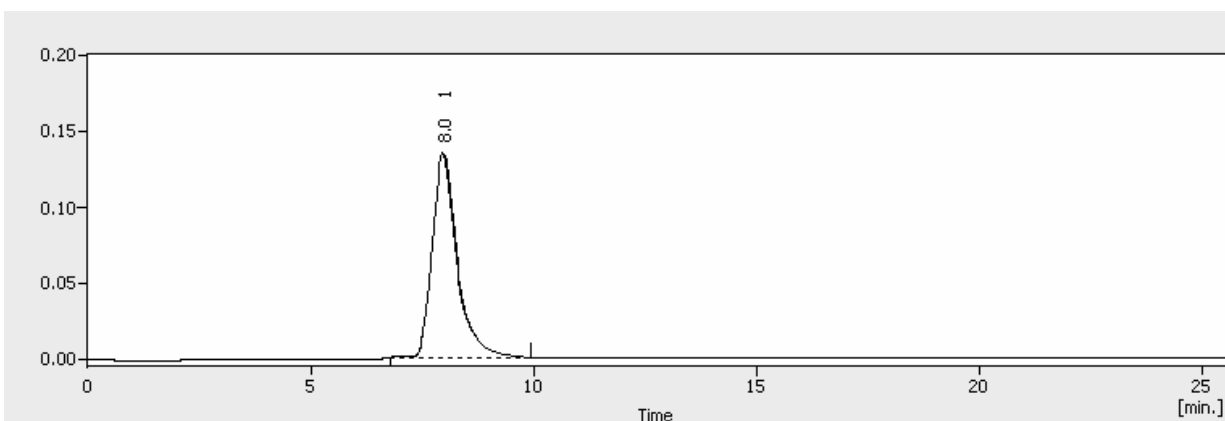


Figure 1: RP-HPLC Chromatogram of standard Piperine at 343 nm

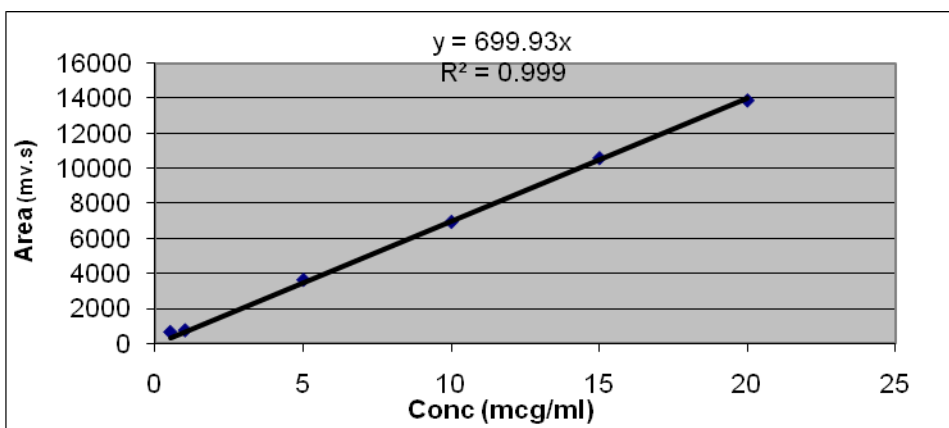


Figure 2: Calibration curve of standard Piperine

Validation parameters

Selectivity and peak purity

Selectivity was checked by using prepared solutions of PC and available standards optimizing separation and detection. The purity of the peaks was checked by multivariate analysis. The three spectra corresponding to up slope, apex and down slope of each peak were computer normalized and super imposed. Peaks were considered pure when there was a coincidence between the three spectra (match factor was =98%).(Table-I)

Linearity, limits of detection and quantification

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amounts of each standard, measured in μg , and the area of the corresponding peak on the chromatogram. Linearity was also confirmed for PC prepared sample solutions. After chromatographic separation, the peak areas obtained were plotted against concentrations by linear regression. Limits of detection and quantification were determined by calculation of the signal-to-noise ratio. Signal-to-noise ratios 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively, of the method.

Precision

The repeatability of the injection integration was determined for both standard piperine and the content of piperine in Pippli churna. A standard solution containing reference compounds and prepared sample solutions was injected. Pippli churna samples were also prepared 2 times to evaluate the repeatability of the process. The mean amount and R.S.D. values were calculated. The precision was calculated at two different concentrations high and low tested in the

concentration range. For standardization the sample was injected at eight different concentrations and linearity was noted [Table-1].

Accuracy

The accuracy of the method was determined by analyzing the percentage of recovery of the piperine in the Pippli churna. The samples were spiked with two different amounts (100, 150 μg) of standard compounds before sample preparation. The spiked samples were extracted by triplicate and analyzed under the previously established optimal conditions. The obtained average contents of the target compounds were used as the "real values" to calculate the spike recoveries [Table-1].

Robustness

For the determination of the method's robustness a number of chromatographic parameters, such as column package and size, mobile phase composition and gradient ratio, flow rate and detection wavelength, were varied to determine their influence on the quantitative analysis. Interday and intraday variability was studied for the sample, by injecting the same concentration of the sample on three different days and the standard error mean was calculated.

RESULTS AND DISCUSSION

In the present study, spectral and chromatographic studies were performed. Results of the The RP-HPLC analyses of PC were performed, samples were injected at seven different concentrations and the linearity was observed with in the concentration range of 0.5-20 $\mu\text{g/ml}$ [Fig. 2].Both Piperine was well separated at retention time 8.020 respectively. The concentration of Piperine present in raw material was found to be 1.41 ± 0.62 w/w in Piper longum fruits (pippli). The content of

Piperine in laboratory formulations (PC-I, II, III) were found to be 0.16 ± 0.002 , 0.19 ± 0.006 , and 0.18 ± 0.004 respectively and in different marketed formulations of PC were, for PC-A (0.15 ± 0.002 %), PC-B (0.16 ± 0.004 %), PC-C (0.17 ± 0.008 %) w/w respectively [Table-2, Fig-1]. The HPLC method was validated by defining the linearity, peak purity, limit of quantification and detection, precision, accuracy, specificity and robustness. For the qualitative purposes, the method was evaluated by taking into account the precision in the retention time, peak purity, and selectivity of piperine elutes. A high repeatability in the retention time was obtained with (R.S.D.) value lower than 1.5% for both standard and samples even at higher concentration. (Table-3). The peak purity was studied in the major peaks. Linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision were evaluated for quantitative purposes [Table-1]. Thus LOD and LOQ found to be 0.063, 0.071 mg/ml respectively which suggest full capacity for quantification of piperine content in different laboratory and marketed batches of PC. R^2 value for the regression equation of the Piperine was higher than 0.9988 thus confirm the linearity of the method. The recovery study was performed at two levels by adding known amount (100, 150 μ g/ml) of piperine with reanalyzed sample of PC found to be close to 99.48 (mean)% and a higher repeatability indicate a satisfactory

accuracy in the proposed methods [Table-3]. Finally the robustness of the method was also assessed. Minor modification of the initial mobile phase gradient (from 25 to 30% solvent instead of 31%) had no effect on the peak resolution of the compound. Therefore, this HPLC method for fingerprinting of PC can be regarded as selective, accurate, precise, and robust. The method is very adaptable because of the precision and repeatability for the traditional Ayurvedic formulation like PC and suitable for routine analysis of Piperine in PC. There was not much variation in the interday and intraday injections performed with the R.S.D. value was found to be 0.080% with the mean standard error 0.03% respectively. Piperine estimation can be utilized as a possible analytical marker for fingerprinting of Pippli churna.

CONCLUSION

The developed high performance liquid chromatographic method for estimation of Piperine from Pippli churna could be used as a valuable analytical tool in the routine analysis, to check the batch to batch variation. Estimation of Piperine can be used as one of the appropriate analytical markers for the finger printing.

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Table-I Validation parameter (Mean% ± SD,n=3)

S. no.	parameter	value
1	Absorption maxima	343
2	Bee's law limit	0.5-20ug/ml
3	Regression equation(y=bx+a)	699.9x
4	Intercept(a)	0
5	Slope(b)	699.9
6	Correlation coefficients(r ²)	0.999
7	LOD mg/ml	0.063
8	LOQ mg/ml	0.071
9	Precision (n=6, % RSD)	0.080
10	Accuracy (%)	99.48

Table-2 Estimation of piperine (Mean% ± SD,n=3)

S.no.	name	Piperine content% w/w	
01	piper longum(pippli)	1.41±0.62	
02	PIPLI CHURNA	PC-I	0.16±0.002
03		PC-II	0.19±0.006
04		PC-III	0.18±0.004
05		PC-A	0.15±0.002
06		PC-B	0.16±0.004
07		PC-C	0.17±0.008

Table-3 Recovery study (Mean% ± SD,n=3)

S.no.	Amount of piperine(microgm/ml)			RSD%	SE	Recovery%
	In sample	added	estimated			
01	100	100	198.4±0.14	0.070	0.038	99.2
02	200	150	349.2±0.32	0.091	0.022	99.77
mean				0.080	0.03	99.48

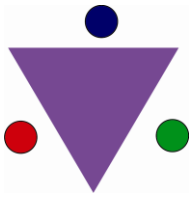
Mean ± SD of six determinations,
SE=Standard error

RSD= Relative standard deviation

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NUTRITIONAL COUNSELLING AND SUPPLEMENTATION: NEED OF THE HOUR IN NATIONAL TUBERCULOSIS CONTROL PROGRAMME IN INDIA

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ABSTRACT

Background: The magnitude of malnutrition is high in India, which easily predisposes the individuals to risk of developing TB and Tuberculosis in turn further aggravates wasting. This vicious cycle results in high morbidity and mortality. Anti TB drug causes nausea, vomiting, poor appetite that further knocks down dietary intake considerably.

Aim: The aim of the present study is to highlight the importance of nutrition in TB patients by assessing the percentage of underweight TB patients and caloric intake using the 24 hour recall method.

Methods and Material: The study was conducted in the urban slum area of Mumbai, covering a population of approximately 72000. Fifty consecutive TB patients who came to DOTS centre were interviewed to assess the dietary intake. Pre-Treatment weight was compared with the standard reference weights of Indian Council of Medical Research's (ICMR). The caloric intake was calculated as percentage of recommended dietary allowance (RDA) for specific age and occupation as per ICMR standards.

Results: Tuberculosis patients interviewed were predominantly females (54%) and in the age group of 20-29 years (42%). The mean age of the patients was 28.56 ± 1.79 yrs. Except for two females, all the TB patients were underweight. The mean weight of the TB patients was 36.82 kilograms. Almost all the TB patients were consuming less than 50% of RDA.

Conclusions: Poor nutritional status predisposes the individual to tuberculosis. It is important therefore that the health care workers while giving priority to drug compliance should not forget to counsel on balanced diet and developing linkages with NGO's to provide food supplementation for filling in the dietary gap, in an effort to improve the nutritional status.

Keywords: Tuberculosis, Thinness, Energy intake, Diet

INTRODUCTION

Tuberculosis (TB) is a global public health problem, responsible for more than 2 million

deaths each year ^[1]. The association between TB and malnutrition is well recognized ^[2], since pre chemotherapeutic era when cod liver oil was given to TB patient to strengthen the host defense ^[3]. Various studies have reported that TB coexists with

malnutrition at the time of starting treatment [4, 5, and 6]. It is well known fact that TB is associated with various socio demographic factors like poverty; poor housing, economic deprivation and these are some of the factors predisposing to poor nutritional status and impaired immune system. Generally malnourished and immunological compromised patients contract TB and TB in turn causes anorexia and wasting. This vicious cycle results in high morbidity and mortality. Also deterioration of nutritional status leads to reactivation of latent TB. Anti TB drug causes nausea, vomiting, poor appetite that further knocks down dietary intake considerably. Randomized controlled trial that provided an energy-protein supplement to TB patients receiving treatment showed gains in lean mass, and greater grip strength so faster recovery^[6].

The magnitude of malnutrition is widely prevalent in India, as evident by several studies that have shown low weight for age amongst under-five, and low BMI for school children, adolescents, and adults. There are several reasons for malnutrition varying from a low birth weight, inadequate caloric intake due to poor socio-economic conditions or dietary fads, occupations involving travelling or odd hours at jobs etc. According to NFHS III 39.5%, 36% and 34% of under-five children, adult women and men respectively are malnourished^[7].

The Revised National TB control programme (RNTCP) using the strategy of Directly Observed Treatment Short course (DOTS) is being implemented throughout India since 2006. The RNTCP quarter I, 2010 report reveal that the case detection rate of New Smear Positive cases is $\geq 70\%$ and treatment success rate of $\geq 85\%$. A total of 3,73,655 TB cases were registered for treatment during the first quarter 2010, of

which nearly 70654 were retreatment cases. In Mumbai itself, RNTCP covers a population of 137 lakhs. The annualized TB case detection cases is 237 per lakh population for Mumbai, which means about 170 TB cases per year^[8].

However the programme does not emphasis on nutritional supplementation or counselling, nor are any efforts made to discuss these aspects by the DOTS strategy. Hence the present study was conducted to highlight the need for incorporating nutrition counselling / supplementation in RNTCP programme with the objective to find out the percentage of TB patients on DOTS who are underweight and assess the nutritional intake of the TB patients through 24 hour recall method.

SUBJECTS AND METHODS

Study settings: The city of Mumbai, called financial capital of India spreads over an area of 437.71 sq. km and is a merger of 7 islands in city area and 4 islands in suburbs. The population of Mumbai is about 1.19 crores with a population density of 27209/sq. km. The city accounts for 1.2% of the Indian population and 12% of the Maharashtra population^[9].

The city of Mumbai is divided into wards for administrative purpose. Each of the wards has several health posts, dispensary, maternity home, post-partum centres and hospitals of BMC to cater to the needs of the community. Apart from the government and the municipal infrastructure there are several private practitioners, private hospitals and charitable trusts catering to the health needs of the Mumbaikars. Health posts are amongst many of the functional DOT centres.

The study was conducted in the urban slum area of Mumbai, Chembur Naka, which falls under the Jurisdiction of the Ramabai health post of M/W ward of the Municipal Corporation of Greater Mumbai (MCGM). The study area has a population of approximately 72000, with an estimated household of 14,400.

MATERIAL AND METHOD

Fifty consecutive TB patients who came to DOTS centre were selected irrespective of their status of TB and treatment and were interviewed using a structured questionnaire. The interviewer collected data on demographic profile, last 24 hr dietary intake and nutritional advice received by them during course of treatment. Other data like sputum results, type of TB, treatment category etc. was recorded from the TB treatment card. Pre-Treatment weight recorded on the TB treatment card was compared with the weight which is used for calculating ICMR recommended dietary intake. Using the 24 hour dietary recall method, dietary intake was expressed in kilocalories (Kcal) per day. It was further calculated as percentage of recommended dietary intake for specific age and occupation as per ICMR standard ^[10].

RESULTS

The TB patients interviewed were mostly in the age group of 20-29 years (42%). Eight of the TB patients were adolescents and two were below the age of 10 years. Four patients were aged 50 years and above. The mean age of the patients was 28.56 ±1.79yrs.

The study population was predominantly females (54%), and amongst the adolescent TB patients again there was

disproportionately higher percentage of females (87.5%) as compared to males (Table 1). The preponderance of females was also found in the age group of 20-29 years. In the age group of 30 and above, the number of male patients was higher than females.

Only 5 of the 50 TB patients were extra-pulmonary of which two was abdominal TB, two lymph node TB and one ileo-caecal TB. Twenty of the TB patients were sputum positive TB. 14% of the TB patients reported of a family member having suffered from TB in the past or has reported a death due to TB. 20% of the patients also gave a past history of TB.

Except for two females in the age group of 20-24 years, all the TB patients were underweight as per the ICMR standards. The mean weight of the TB patients was 36.82 with Standard error of 1.34 (Table 2).

The dietary assessment using the 24 hour recall method revealed that the patient's were consuming less than 50% of their daily caloric intake. The reasons for poor dietary consumption was not only due to socio-economic conditions but also due to loss of appetite, likes and dislikes for different food items, irregular working hours, non-availability of home -made food etc.. Many patients were scared to eat because of nausea and vomiting, which further affected the drug compliance.

DISCUSSION

Nutritional status determines normal health and functioning of all body system including immune system which is responsible for various infectious diseases. General malnutrition compromises cell mediated immunity leading to TB. Though nutrition

has proven importance, the Revised National Tuberculosis Control Programme, does not emphasise nor focus on the nutrition of the TB patients.

96% of the TB patients were undernourished. A study in Malawi and Ghana found that 57% and 51% of the TB patients admitted to the district hospital were having less than 18.5 kg/m².^[11,12] A study in Ajmer in India, found that 75% of the patients had low BMI.^[13]

Dietary intake was less than 50% of RDA in all the patients in the present study which was almost similar to the findings of the study conducted in Ajmer^[13]. Dietary intakes was very low due to poor appetite, likes and dislikes for different food items, irregular working hours, non-availability of home-made food, nausea, vomiting, side effects of anti TB drugs etc. Though TB treatment improves the nutritional status, it is limited to gain in fat mass. Thus even a balanced diet is inadequate to support lean body mass repletion. A majority of the patients (94%) reported that they did not receive nutritional counseling. Since the focus is only on ensuring drug compliance, health workers are counseling on regular intake of drugs and no effort was made to assess the dietary intake and explore reasons for poor weight gain.

Nobody can deny the role of a balanced diet even in normal healthy individuals. Therefore, it is all the more relevant to have a proper diet for a sick person whose rate of metabolism is high and wasting is pronounced. Though no special emphasis on diet is required in the modern management of a TB patient whose general health condition is satisfactory and who is able to take his usual diet, but drugs alone cannot help a patient if his general health condition is poor and is unable to take a proper diet.

While giving priority to drugs, we must not forget the importance of a basic, balanced diet in the management of TB.

In conclusion it may be said that proper drugs, diet, improved living conditions and health awareness all play vital roles in handling the problem of TB. Some recommendations that we want to suggest are

- Nutritional assessment for determination of nutritional status at time of registration of patient.
- Nutritional counseling/education on symptom management and improved dietary intake during/after TB treatment.
- Food supplementation to fill in the dietary gap.

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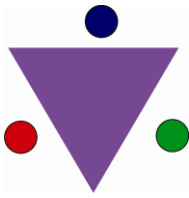
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Table 1: Age and Sex distribution of TB patients

Age Group	No. of females (%age)	No. of males (%age)	Total
<20	8(29.62%)	2(8.69%)	10(20%)
20-29	11(40.74%)	10(43.47%)	21(42%)
30-39	4(14.81%)	6(26.09%)	10(20%)
40-49	2(7.41%)	3(13.04%)	5(10%)
>=50	2(7.41%)	2(8.69%)	4(8%)
Total	27(54%)	23(46%)	50(100%)

Table 2: Nutritional Status of Male and Female Tuberculosis Patients

Sex	UNDERNOURISHED		Total
	N	Y	
F	2	25	27
M	0	23	23
	2	48	50



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ASSOCIATION OF LIFESTYLE PRACTICES AND DIETARY PATTERN WITH CHILDHOOD OBESITY AND THE IMPACT OF NUTRITION EDUCATION

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ABSTRACT

Childhood obesity has increased in both developed and developing countries although the pace and pattern differ from country to country. It has profound public health consequences, as seventy percent of the overweight children become overweight adults. The present study was focused on the prevalence of childhood obesity in selected schools of Kottayam district and to explore the association of obesity with variables like socio economic status, location of residence, birth weight of the child, nutrition during infancy and life style pattern. A total of 2216 children were selected from eight public schools which constitute 1008 boys and 1208 girls in the age group of 10 to 14 years. A well framed questionnaire was distributed to elicit details on demographic profile, life style and food consumption pattern. Height and weight was taken for all the children to identify the BMI and status of body fat. Of the total population 18 percent were obese, 14 percent were overweight, 56 percent were normal weight and 12 percent were underweight. The results of the study exposed the fact that the percentage of overweight and obese children are growing in Kerala also, like in the other states of India and globally. Obesity and overweight were seen more in girls and underweight seemed to be more in boys indicating an increasing trend in the percentage of obesity among girls compared to boys. In rural areas also an increasing trend of overweight and obesity was observed although underweight children are still prominent. So it was concluded that the increasing trend of the modern day epidemic of overweight and obesity in children calls for immediate action to reduce the incidence through appropriate nutrition intervention programmes involving school children, their parents and school authorities. If immediate measures are not taken the condition can lead to serious problems beyond repair.

Key words: Body Mass Index, obesity, nutrition education.

INTRODUCTION

Childhood obesity is an emerging pandemic of the new millennium. This has profound public health consequences, as 70 percent of

overweight children become overweight adults (Sanjeev, 2009). According to Swaminathan (2005) a person whose body weight is higher than normal by 15-20 percent is considered as overweight and by 25 percent is considered as obese. Obesity is a major risk factor for many chronic

diseases, such as cardiovascular disease and diabetes. Moreover overweight and obesity exacerbate many chronic diseases. Obesity is a complex disease influenced by genetic and environment factors and their interactions. It is a major risk factor for metabolic diseases, each of which is influenced by their own specific genes and environmental factors (Butte, 2006). Obese children tend to be more isolated and have lower self-esteem than their peers. A systematic review of studies on the relationship between physical activity in children and obesity found that roughly half had no effect and the balance had a negative effect (that is increased physical activity level were protected). Many cross-sectional studies have looked at the association between television viewing and childhood obesity. Some found only a weak association, but most found a positive association in children all over the world. Snacking is gaining prominence as a potential risk factor for obesity as is skipping meals. Those who do not consume breakfast tend to eat a large amount of food in the evening, and this imbalance could lead to a higher risk of obesity. It has been shown that family structure including family size, birth order of the child as well as whether it is a single or joint parent family may have an effect on childhood obesity (Wang et al, 2007).

During the past two decades, the prevalence of obesity in children has risen greatly worldwide and this excessive fatness has arguably become a major health problem of both developed and developing countries. Overweight and obesity during childhood is a matter of growing concern in India also. Most individuals develop their eating and activity patterns during childhood. The transition in nutrition and life style by the

popularity of fast foods, soft drinks, sedentary life style, lack of exercise, increased television watching and computer use are the common trends adopted by children today. These may be the causes of overweight seen in children of both rural and urban areas (Ramachandran, 2002). Kerala has made remarkable achievement on par with the developed countries in the field of women and children's health during the last few decades. However, overweight and obesity is a growing health concern in Kerala too; the consequences of which can cause disaster to the future generation (Geetha, 2003). Considering the threats of overweight and obesity in this cyber era, the present study is carried out in selected schools of Kottayam educational district among children between the age group of 10 to 14 years to see the extent of overweight, obesity and underweight among the children of Kerala. Keeping these in mind the study has been focused with the following objectives to:

- Identify the prevalence of obesity in selected schools of Kottayam district and study the socio economic status of selected school children,
- Explore the association of obesity with variables such as socio- economic status, infant nutrition, life style pattern, dietary habits and
- Find out the impact of nutrition education to the parents of obese children.

MATERIALS AND METHODS

Selection of Area:

Considering the good response, ease of communication and familiarity of the area the researcher selected eight private schools in Kottayam district of Kerala.

Selection of Sample:

A total of 2216 children both male and female in the age group of 10-14 years were selected for the study. Of these 1008 were boys and 1208 were girls. Since the age group selected for the study ranged from 10-14 years, all the students studying from 5th standard to 9th standard were selected for the study.

Conduct of Study:

In order to fulfill the objectives of the study a questionnaire was formulated to elicit the background information of the children. Anthropometric measurements were taken to identify the BMI and the status of body fat. A twenty four hour recall dietary survey was conducted for three consecutive days to understand the food consumption pattern of the selected children. Nutrition education was imparted to the parents of obese children during a Parent Teacher Association meeting conducted by the school authorities. Pamphlets, booklet and power point presentation were developed to impart nutrition education.

Formulation of questionnaire:

The framed questionnaire embracing the details of demographic data, monthly income, monthly expenditure pattern, dietary habits, details of food expenditure, consumption of junk foods, duration of television watching, extracurricular activities, duration of indoor and outdoor games, physical activity pattern, type and frequency of snacks and beverage consumption, food preferences of the subjects, type and amount of oil used in the family and heredity of obesity in the family and feeding pattern during infancy was formulated.

Anthropometric measurement of the subjects:

The height and weight of the subjects were taken by the standard procedure. From the recorded weight and height of the subjects, Body Mass Index (BMI) was calculated.

Body Mass Index (BMI):

Assessing pediatric obesity is not as straight forward as it may seem, but there is now a consensus that Body Mass Index (BMI) should be used for clinical practice and epidemiology. BMI values in children are much lower than in adults, and BMI changes with age. So BMI cutoffs to define obesity in adults are not appropriate for children. National BMI reference data are now available and are widely used and recommended.

>95th percentiles : **obesity**
86th -95th percentiles : **over weight**
5th -85th percentiles : **normal weight**
<5th percentiles : **under weight**

Designing a pamphlet, booklet and power point

A pamphlet, booklet and power point presentation was prepared on obesity focusing on its causes, complications, remedial measures, the importance of diet and behavior modifications in preventing obesity.

Administration of Nutrition Education

Nutrition education was administered by the following procedures.

Assessment of nutritional knowledge:

A pretest was conducted to test the nutritional knowledge of the parents of the selected obese children with special reference to functions and sources of nutrients and dietary pattern. The parents were asked to state true or false for the given questions. Every correct answer was given one mark and the scores were summed up.

Imparting Nutrition Education:

A study on the dietary pattern of children and the nutritional knowledge of the parents was carried out. The tools developed for education included a pamphlet, booklet and power point presentation.

Impact of Nutrition Education

The impact of nutrition education given by the investigator was analyzed after two months using questionnaire.

RESULTS

Food consumption in front of television

Most of the children prefer to have food in front of television. Table 1 shows the list of children who consumed food while viewing television.

Table 1 Food consumption and viewing television

Table 1 shows that when 17 percent of the obese subjects consumed food in front of the television, 6 percent of the normal weight samples did not. Normally the subjects consumed snacks and tiffin items in front of television. It was reported that children who viewed television consumed high fat food and fast food, drink more soft drinks and consumed fewer fruits and vegetables.

Type of play

Table 2 gives details about the type of play in which the selected samples were engaged.

Table 2 Type of play involved by the samples

Table 2 shows that when 14 percent of the obese subjects were interested in indoor games only 4 percent preferred outdoor games. It was also noted that 20 percent of the normal weight subjects preferred outdoor games and 5 percent of the underweight subjects preferred indoor games. Although the increase in childhood obesity is frequently attributed to a decline

in physical activity and remarkable lack of consistency exists in the relation between level of physical activity and degree of fatness.

Family history of obesity

The details about the history of obesity in the family of the selected children is depicted in table 3

Table 3 Family history of obesity

Table 3 reveals the fact that 3 percent of the obese children's and 4 percent of the overweight subject's mothers were obese. Two percent each of obese, over weight and normal weight subject's fathers were obese. However 69 percent of the family members were found to be free from heredity factor for obesity. Studies have shown that the likelihood that a child will become obese in adulthood is markedly increased if either his or her parents are obese.

BMI of the subjects:

Body Mass Index (BMI) of the selected children is shown in table 4.

Table 4 BMI of the subjects

Table 4 clearly shows the fact that out of the 2216 children selected, 18 percent were obese and 14 percent were overweight. Fifty six percent and 12 percent of the selected children were of normal weight and underweight respectively. It was also noted that most of the obese subjects were from affluent families.

BMI of children with different food habits.

The details regarding the BMI of children with different food habits is depicted in table 5

Table 5 BMI of children with different food habits

From table 5 it was observed that 13 percent of the obese subjects were non vegetarians. It was also interesting to note that only two percent of the obese samples were vegetarians, while 37 percent of the normal weight children and seven percent of the underweight children were non vegetarian. Sanjeev (2009) from his studies also showed that the crowd in the restaurants and fast food centers can reveal the changing pattern of food intake among teenagers.

Feeding practices of the subject during infancy.

The details regarding the feeding practices of the subject during infancy is elicited in table 6.

Table 6 Feeding practices during infancy

It was clear that 5 percent of the obese children and 3 percent of the overweight children were not breast fed during infancy. This was because either due to the death or due to some illness of the mother. Ten percent of the breast fed subjects was under weight and 53 percent were normal weight. Breast feeding protects against obesity.

Parents' awareness about nutritional aspects

Table 7 gives a clear picture of the scores secured by parents of overweight and obese subjects in relation to various nutritional aspects.

Table 7 Parents awareness about nutritional aspects (N=567)

It was seen that before nutrition education 39 percent scored less than 10 marks and 47 percent scored between 10 and 20. Only a few percentages of 14 scored more than 20 marks. This clearly bring out the fact that majority of the parents were unaware about

nutritional aspects. After nutrition education 56 percent of the parents scored between 20 to 25 marks. This indicated that there was a rapid rise in the nutritional knowledge of the parents. Obesity is easier to prevent than to treat and prevention focuses in large measure on parent education. In childhood, parent education should center on proper nutrition, selection of low fat snacks good exercise or activity habits and monitoring on television viewing.

DISCUSSION

The present study was conducted at eight schools of Kottayam district, to identify the prevalence of obesity in selected schools and to explore the association of obesity with other variables such as socio-economic status, location of residence, birth weight of the child, birth order of the child and feeding habits during infancy. A total of 2216 children were selected from eight public schools which constitute 1008 boys and 1208 girls in the age group of 10 to 14 years. A well framed questionnaire was distributed to the children to elicit details on demographic profile, life style pattern, food consumption pattern, prevalence of obesity among the children, association of obesity with different variables and their nutritional knowledge. Height and weight for all the selected children were taken. Nutrition education was imparted to the parents of obese children during a Parent Teacher Association meeting conducted by the school authorities. Pamphlet and booklet was developed which was distributed to the parents. A power point presentation was also made incorporating all the necessary details regarding obesity. The results of the study exposed the fact that the percentage of overweight and obese children are increasing in Kerala also, like in other states

of India and globally. The study also showed that when obesity and overweight were seen more in girls and underweight seemed to be more in boys indicating an increasing trend in the percentage of obesity among girls compared to boys. In short the study showed an increasing trend of overweight in children particularly in girls of urban areas. In rural areas also an increasing trend of overweight and obesity was observed although underweight children are still prominent.

CONCLUSION

Childhood obesity is one among the primary priority programs of World Health Organization and is the most serious public health challenge of the twenty first century. The problem is global and is steadily affecting many low and middle income countries, particularly in the urban settings. It was concluded that the increasing trend of the modern day epidemic of overweight and obesity in children calls for immediate action to reduce the incidence through appropriate nutritional intervention programmes involving school children, their parents and school authorities. If immediate measures are not taken the condition can lead to serious problems beyond repair.

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Table 1 Food consumption and viewing television

Food consumption	BMI GRADES (N= 2216)							
	Underweight		Normal weight		Overweight		Obese	
	No	%	No	%	No	%	No	%
Consume food	248	11	1094	49	287	13	370	17
Do not consume food	17	1	137	6	21	1	42	2
Total	265	12	1231	56	308	14	412	18

Table 2 Type of play involved by the samples

Type of play	BMI GRADES (N= 2216)							
	Underweight		Normal weight		Overweight		Obese	
	No	%	No	%	No	%	No	%
Indoor games	112	5	781	36	227	10	316	14
Outdoor games	153	7	450	20	81	4	96	4
Total	265	12	1231	56	308	14	412	18

Table 3 Family history of obesity

Relation	BMI GRADES (N= 2216)							
	Underweight		Normal weight		Overweight		Obese	
	No	%	No	%	No	%	No	%
Father	8	-	41	2	39	2	49	3
Mother	12	1	173	8	87	4	67	3
Siblings	11	1	39	2	27	1	13	1
Grand parents	17	1	64	3	18	1	9	1
No history	217	10	914	41	137	6	274	12
Total	265	12	1231	56	308	14	412	18

Table 4 BMI of the subjects

BMI GRADES	Underweight	Normal weight	Overweight	Obesity
Number	265	1231	308	412
Percent	12	56	14	18
Total	12	56	14	18

Table 5 BMI of children with different food habits

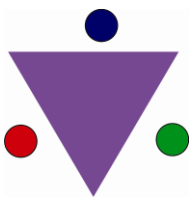
Types of diet	BMI GRADES (N= 2216)							
	Underweight		Normal weight		Overweight		Obese	
	No	%	No	%	No	%	No	%
Vegetarian	61	3	112	5	39	2	58	3
Non vegetarian	157	7	814	37	242	11	282	13
Ova vegetarian	47	2	305	14	27	1	70	3
Total	265	12	1231	56	308	14	412	18

Table 6 Feeding practices during infancy

Feeding practices	BMI GRADES (N= 2216)							
	Underweight		Normal weight		Overweight		Obese	
	No	%	No	%	No	%	No	%
Breast fed	213	10	1171	53	241	11	294	13
Not breast fed	52	2	60	3	67	3	118	5
Total	265	12	1231	56	308	14	412	18

Table 7 Parents awareness about nutritional aspects (N=567)

Scores	< 10	%	10 to 20	%	20 to 25	%
Before education	219	39	267	47	81	14
After education	112	20	136	24	319	56



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PRODRUGS: AN APPROACH TOWARDS BETTER TARGETTING

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ABSTRACT

Prodrugs, the pharmacologically inactive derivatives of active drugs, are designed to maximize the amount of active drug that reaches its site of action, through manipulation of the physicochemical, biopharmaceutical or pharmacokinetic properties of the drug. But new developments are increasingly taking the concept beyond issues of availability to include targeting. The development of prodrugs promises to be very effective method for treatment of diseases in future. This approach has several advantages over conventional drug administration. In this mini review, prodrugs are discussed with a focus on the viability of the prodrug approach as a means of attaining targeted drug delivery. Next, several examples of where the prodrug approach has been used to achieve targeted delivery will be discussed.

Key words: prodrugs, targeting, targeted drug delivery.

INTRODUCTION

A drug can be defined as a chemical used for treating, curing or preventing disease in human beings or in animals. In the process of treatment, drugs are also used for medical diagnosis and for restoring, correcting, or modifying physiological functions. Conventional drugs suffer from many drawbacks in their performance like site specificity, permeability and resistance. Almost all drugs possess some undesirable physicochemical and biological properties. Their therapeutic efficacy can be improved by minimizing or eliminating the undesirable properties while retaining the

desirable ones ¹. This can be achieved through following means.

- The biological approach is to alter the route of administration which may or may not be acceptable to patient.
- The physical approach is to modify the design of dosage form such as controlled drug delivery of drug.
- The third and best approach in enhancing drug selectivity while minimizing toxicity is the chemical approach for design of prodrugs ².

The Prodrug Concept

Albert and his coworkers were the first ones to suggest the concept of prodrug approach for increasing the efficiency of drugs in 1950. They described prodrugs as pharmacologically inactive chemical

derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness and/or to decrease associated toxicity². Subsequently such drug-derivatives have also been called 'latentiated drugs', 'bioreversible derivatives', and 'congeners', but 'prodrug' is now the most commonly accepted term. Thus, prodrug can be defined as a drug derivative that undergoes biotransformation enzymatically or nonenzymatically, inside the body before exhibiting its therapeutic effect. According to IUPAC (International Union of pure and applied chemistry): Prodrug is defined as any compound that undergoes biotransformation before exhibiting its pharmacological effects³.

Characteristics of a Prodrug

In recent years numerous prodrugs have been designed and developed to overcome barriers to drug utilization such as⁴:

- Low oral absorption properties
- Lack of site specificity
- Chemical instability
- Toxicity
- Bad taste
- Bad odour
- Pain at application site^{4,5}

Ideal properties of prodrug

It should have intrinsic pharmacological activity that means it should not change receptor configuration that is necessary for pharmacological response. It should rapidly transform into the active form where desired and metabolic fragment, apart from the active drug should be nontoxic⁶.

Limitation of prodrug⁷

The problem associated with prodrug design is its toxicity which is due to

- Formation of unexpected metabolite from the total drug conjugates.
- Toxicity may be due to inert carrier generated by cleavage of moiety and drug conjugate which is converted into toxic metabolite.
- The prodrug might consume a vital cell constituent such as glutathione during its activation stage which causes depletion of prodrug⁸.

Classification of Prodrugs

A) Carrier linked prodrug

Contain a group that can be easily removed enzymatically (such as ester) to reveal the true drug as shown in fig.1. Ideally the group removed is pharmacologically inactive and nontoxic while the connecting bond must be labile for efficient activation in vivo. Prodrugs are the ones where the active drug is covalently linked to an inert carrier transport moiety. They are generally esters or amides. Such prodrugs have greatly modified lipophilicity due to the attached carrier and the active drug is released by hydrolytic cleavage, either chemically or enzymically⁹. It can be further subdivided into-

Bipartate- Composed of one carrier (group) attached to the drug.

Tripartate- Carrier group is attached via linker to drug.

Mutual Prodrugs- Two drugs linked together.

B) Bio precursors

Metabolized into a new compound that may itself be active or further metabolized to an active metabolite as shown in fig.2 (e.g. amine to aldehyde to carboxylic acid)¹⁰.

PRODRUG MEDIATED TARGETED DELIVERY

Targeting viruses

The topic of targeted delivery of antiviral agents has been recently reviewed¹¹. One of the best and simplest examples of targeted drug delivery via a prodrug approach is the antiviral agent. Acyclovir (9-(2-hydroxyethoxymethyl) - guanine). As shown in Fig. 3, its targeting is achieved primarily by site-selective activation. The herpes virus encoded enzyme, pyrimidine deoxynucleoside (thymidine) kinase, is responsible for converting acyclovir to its phosphate monoester. Subsequently, cellular enzymes catalyse the conversion of the monoester to the di- then triphosphorylated species. This latter species is the pharmacologically active one, and the enzymatic conversion to triester occurs to a significantly greater extent in the herpes-infected cells. Because of its site-specific activation, acyclovir displays a high therapeutic activity against herpes virus, essentially no activity against adenovirus, minimal metabolic degradation following systemic administration, and very low toxicity against uninfected host cells^{12, 13}.

Targeting the colon

A fundamentally simple example of targeting which exploits both site-specific transport and activation is the colon-specific delivery of drugs illustrated in Fig. 4. With this approach, prodrugs are formed by coupling the drug to a hydrophilizing moiety that is susceptible to cleavage by enzymes secreted by the bacterial microflora associated with the lower gastrointestinal tract¹⁴. Following oral administration of the prodrug, drug absorption in the stomach and small intestines is decreased due to the polar nature of the moiety; therefore, greater levels of the drug, in the form of the prodrug, can reach the colon. Within the colon, the bacterially derived enzymes catalyze the conversion of the prodrug to the

more lipophilic drug which is now available for absorption through the colonic membrane. This targeting concept has been recently reviewed. Glycosidic and glucuronidic prodrugs, of agents such as dexamethasone, naloxone, and menthol, that exploit bacterial glycosidases and glucuronidases have been studied for their colon-targeting potential¹⁵.

Targeting the kidney

Site-selective prodrug activation, by exploiting the relatively high response site activity of an enzyme, has also been achieved with targeted delivery to the kidney. For example, dopamine was found to selectively accumulate in the kidney following the intraperitoneal administration of the double prodrug, γ -glutamyl-L-dopa, to mice¹⁶. As shown in Fig. 5, the prodrug is activated by the sequential catalytic actions of two enzymes that possess high activity in the kidney. First, γ -glutamyl transpeptidase catalyzes the cleavage of the γ -glutamyl linkage: the L-dopa which is formed is then decarboxylated to dopamine by L-amino acid decarboxylase. The end result is that dopamine is more readily available to exert its therapeutic effect (i.e., renal vasodilation) at the response site while causing less effects at non-response sites (as was demonstrated by an unchanged systemic blood pressure).

Targeting the liver

To achieve targeted drug delivery to the liver, researchers have attempted to exploit site-selective transport pathways. One such pathway is the bile acid transport system associated with the sinusoidal membrane of hepatocytes¹⁷. The bile acid prodrugs of several compounds, such as chlorambucil, thyroid hormone (L-T), and HMG-CoA reductase inhibitors¹⁸ have demonstrated some degree of hepatic targeting.

Targeting the brain

To preferentially deliver amine-containing drugs to the brain¹⁹ have developed a prodrug delivery system which exploits the oxidative conversion of a dihydropyridine promoiety to its corresponding pyridinium salt (Fig. 6). With this approach, an amine-containing drug is coupled to a lipophilic dihydropyridine promoiety that facilitates the penetration of the drug, in the form of the prodrug, through the blood-brain barrier (BBB). Oxidation of the dihydropyridine functionality in the peripheral compartments results in the formation of the corresponding polar pyridinium salt which can be readily excreted from the body, whereas formation of the pyridinium salt in the CNS results in site retention because of the poor permeation characteristics of the charged species and the permeability characteristics of the BBB. Subsequent cleavage of the pyridinium salt promoiety releases the drug which is now present at elevated levels within the brain. The proof-of concept has been demonstrated for a wide variety of amine-containing drugs.

Targeting with antibodies

Selective activation of a prodrug of an anticancer agent at tumor sites is severely limited by the commonality of enzymes associated with normal and neoplastic tissues. Therefore, two approaches utilizing monoclonal antibodies²⁰ have been studied as a way of selectively activating a prodrug at the tumor site. These approaches, which have received considerable attention over the last decade, are Antibody- Drug Conjugates and Antibody-Directed Enzyme Prodrug Therapy (ADEPT). Antibody-drug conjugates (immune conjugates) are macromolecular prodrugs that are formed by covalently linking cytotoxic agents to monoclonal

antibodies reactive with tumor associated antigens. A number of chemical coupling methods have been utilized to produce the drug-antibody conjugates, and several tumor-associated antigens have been identified, and the respective monoclonal antibodies have been produced. In order for targeting to be achieved, the drug must be cleaved from the antibody after the immune conjugate binds to a tumor cell; this usually occurs intracellularly (e.g. lysosomal degradation) after internalization of the conjugate Fig. 7a shows a simplified depiction of this process²¹.

Antibody-directed enzyme prodrug therapy (ADEPT)

A simplified scheme of the ADEPT approach to targeted drug delivery is shown in Fig. 7b. Ideally, administration of an enzyme, which is covalently linked to a monoclonal antibody, binds selectively to the respective tumor-associated antigen. After the antibody-enzyme conjugate (Ab-E) has localized within the tumor and has been cleared from non-target sites, a prodrug, that is a substrate for the enzyme, is administered. Upon contact with the targeted enzyme, the prodrug is converted to the drug at the tumor site. This targeting approach has been well reviewed. An important dosing consideration with the ADEPT approach is optimizing the time interval between administration of the Ab-E and the prodrug. To attain adequate tumor uptake, high plasma and extracellular fluid levels of the Ab-E should typically be maintained for several hours^{22, 23}. Once adequate tumor levels are achieved (and prior to prodrug administration), sufficient time should be allowed for significant plasma clearance or inactivation of the non-tumor-associated Ab-E to minimize prodrug activation at non tumor sites (a potential source of toxicity).

CONCLUSION

The area of prodrug-mediated targeted drug delivery has made many strides within the last decade; however, there is still a great need for additional work to further improve existing approaches and to develop newer ones. At present prodrug are not prevalent in clinical use, in future there will be prodrugs for every known drug to make them effective in treatment. Drug discovery and prodrug development appear to be complementary for the generation of target specific medicines of future. The sole use of a prodrug to achieve targeted drug delivery is limited unless the target site possesses a

unique enzyme system for activating the prodrug (as in the case of acyclovir). Hence, the combination of a prodrug approach with an additional approach which facilitates the targeting, as exemplified by the ADEPT. At present the research in this area is at a nascent stage due to lack of information, regarding all enzymes or receptors, most suitable for targeting purposes. As the unrevealing of the microbiological details of affected targets become clear, prodrug development will surely decrease side/toxic effect of drugs and also trigger development of more potent primary drugs.



Fig.1. Carrier linked prodrugs.



Fig.2. Bio precursor.

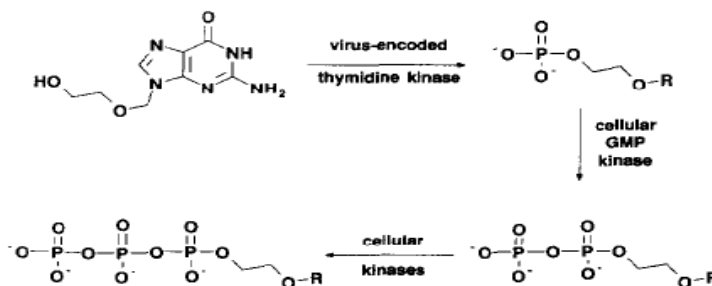


Fig.3. Bioconversion of the antiviral agent acyclovir, to the pharmacologically active triphosphate ester⁷ the site –selective activation arises from the catalytic involvement of herpes virus – encodes thymidine kinase.

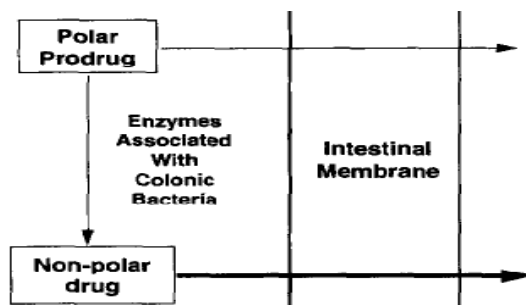


Fig.4. Scheme proposed by Friend and Chang¹⁰ For prodrug- mediated targeted drug delivery to the colon by the use of glycosidic and glucuronic prodrugs.

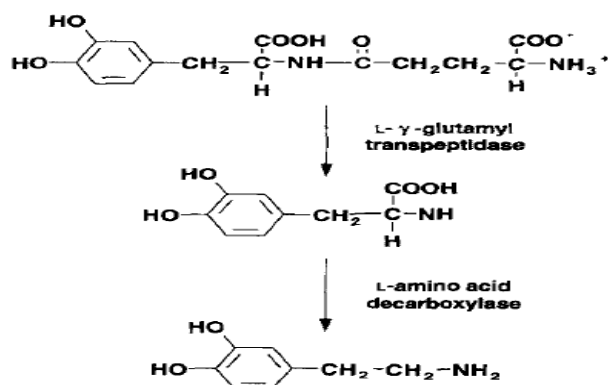


Fig.5. Kidney selective delivery of dopamine via the administration of L-γ-glutamyl-L-dopa.

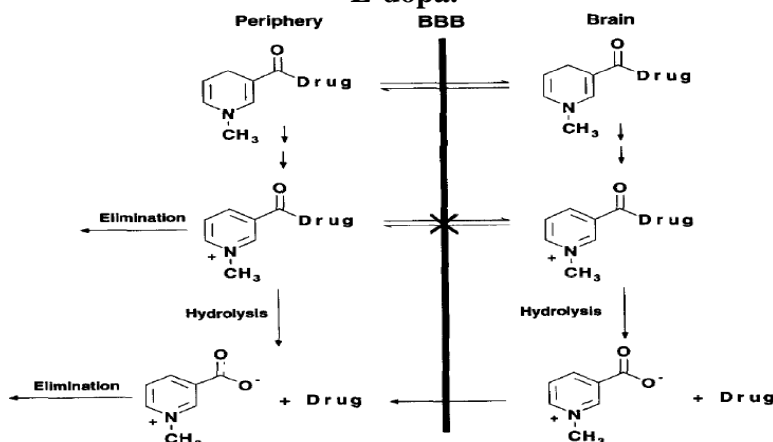


Fig.6. The redox based, prodrug- mediated, brain targeting system for amine containing drugs.

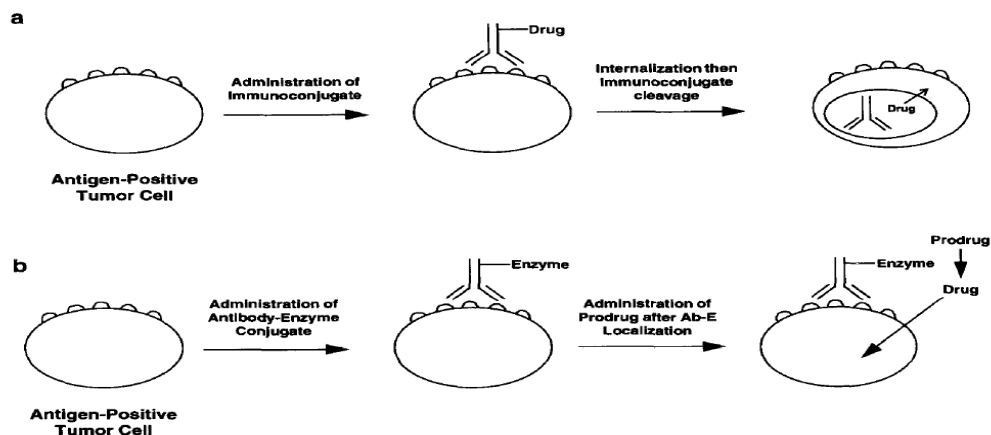
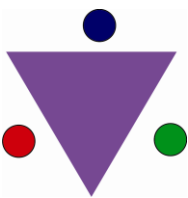


Fig.7. (a) simplified scheme of the site-selective delivery and activation of a monoclonal antibody-drug conjugate to a tumor cell. (b) Simplified scheme of the site-selective activation of a prodrug at a tumor cell using the ADEPT approach²³.

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ANTIBACTERIAL PROPERTIES OF EXTRACTS IN CYANOBACTERIA ISOLATED FROM COASTAL REGION OF ORISSA

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ABSTRACT

Biologically active substances were reported from cyanobacteria. Many strains of cyanobacteria (blue green algae) are known to produce intracellular and extracellular secondary metabolites with diverse biological activity such as antibacterial, antifungal, antiviral and antineoplastic properties. In the present study attempt has been made to assess the antibacterial effect of aqueous and methanol extracts of six marine cyanobacteria belonging to the genus *Phormidium* and *Lyngbya* isolated from coastal region of Orissa against some Gram positive and Gram negative human pathogenic bacteria following Disc diffusion assay method. The results showed that all the test species of cyanobacteria exhibited antibacterial activity on aqueous and methanolic extracts. However the intensity of antibacterial properties varied among the pathogenic strains of bacteria used for the experimental purpose. Among the six cyanobacterial species the antibacterial effect was found to be significant in *Phormidium tenue* and *Lyngbya maintensiana* as compared to other test species. This shows greater biotechnological potential of these two species which needs further studies.

Key words: Aqueous, Cyanobacteria, Orissa coast, Secondary metabolites.

INTRODUCTION

Cyanobacteria are very old group of prokaryotic organism and relics of the oldest photoautotrophic vegetation in the World that occurs in fresh water, marine and terrestrial habitats. They vary from small single celled forms to complex multicellular forms. Cyanobacteria have drawn much attention as prospective and rich sources of

biologically active constituent and have been identified as one of the most promising groups of organism to be able to produce bioactive compounds. Cyanobacteria occur in varied habitats ranging from marine to fresh water, from desert sand to hot springs and from snow field to ice caps. There are several reports of cyanobacterial compounds possessing a wide range of biological activity such as antibacterial, antiviral, antineoplastic effect (Finical and Paul 1984, Hodgson 1984, Ballesteros *et al.*, 1992, Bhosale *et al.*, 2002). However the

production capacity of antimicrobial substances by some species varies (Pesando, 1990). Screening of cyanobacteria for antibiotics and other pharmacologically active compound has received increasing interest as potential sources of new drugs (Fish and Codd 1994, Borowitzka 1995, Ostensvik *et al.*, 1998, Schlegel *et al.*, 1999). The purpose of the present work was to evaluate the antibacterial activity of six strains of cyanobacteria viz. *Phormidium tenue*, *Phormidium mole*, *Phormidium angustissium*, *Phormidium bohneri*, *Lyngbya maintensiana*, *Lyngbya chaetomorphae*, against Gram positive bacteria, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus brevis*, *Bacillus subtilis*, *Streptococcus aureus* and Gram negative bacteria *Escherichia coli* and *Shigella fleximinia*. So far studies on biologically active substances from cyanobacteria isolates from Orissa coast has not been attempted. Hence the present study may provide valuable information for biological exploitation of cyanobacteria from Orissa coast for industrial applications.

MATERIALS AND METHODS

Algal samples were collected from different locations along Orissa coast during the month of March to June 2004 (fig-1). Epiphytic and extraneous matters were removed by washing first in sea water and then in the fresh water. The samples were transported to the laboratory in the sterile polythene bags at ice temperature. The algal samples were separated into unialgal condition by repeated subculturing in

enrichment media in both liquid and agar slants and were maintained at $26 \pm 1^{\circ}\text{C}$ and 4000 lux light intensity with a photoperiod of 16h. light and 8h dark. Identification was done using morphological variation studies and taxonomical approaches according to Desikachary (1959). Altogether six species so far identified were taken as test organisms such as *Phormidium tenue* (Menegh) Gomont; *Lyngbya maintensiana* (Menegh) Gomont; *Phormidium mole* (Kutz) Gomont; *Phormidium angustissium* (W. et. G. S. West); *Phormidium bohneri* (Schmidle); *Lyngbya chaetomorphae* (Iyengar et. Desikachary). The algal samples were shade dried, cut into small pieces and powdered in a mixture grinder. The extractions were carried out in solvents such as methanol and water (aqueous). These two extracts were tested for antimicrobial property on five Gram positive bacteria, *Staphylococcus epidermidis* (MTCC1114); *Staphylococcus aureus* (MTCC 25923); *Bacillus bravis* (MTCC 6853); *Bacillus subtilis* (MTCC 6633); *Streptococcus aureus* (MTCC3542), and two Gram negative bacteria, *Escherichia coli* (MTCC 11230) and *Shigella fleximinia* (MTCC23223). The antibacterial assay was done following disc diffusion assay method (Casida 1986). 500 μg of each extracts (50 μl) dissolved in sterile filter paper disc (6mm) was used. After evaporation of the solvent the disc were placed in nutrient agar containing test pathogens. The plates were then incubated overnight at 37°C and observations were made after 48h of incubation.



Figure.1-Map showing the collection sites of cyanobacteria used for experimental purpose.
Latitude- 17^o49'N to 22^o34'N Longitude- 81^o27'E to 87^o27'E

RESULTS AND DISCUSSION

The results of antibacterial activity of six marine cyanobacteria isolated from coastal region of Orissa to different pathogenic strains of bacteria are represented in table-1. The result showed that all the test organisms possesses antibacterial property against all the strains of bacteria in both aqueous and methanolic extracts. However their intensity varies among strains basing on type of pathogenic bacteria and the extracts type. In this study it was observed that the test species of marine cyanobacteria *Phormidium tenue* and *Lyngbya maintensiana* showed significant antibacterial activity as compared to other four species. It is evident from the study that

aqueous extracts of *Phormidium tenue* showed prominent antibacterial activity with inhibition zone size of 18.8mm diameter against *Staphylococcus epidermis* followed by inhibition zone of 17.6mm against *E.coli*. For other pathogenic strains of bacteria the size of inhibition zone ranges from 9 – 14mm. Compared to aqueous extracts, methanolic extracts showed size of inhibition zone ranging from 8-15mm against the test pathogenic Gram positive and Gram negative bacteria. Similarly *Lyngbya maintensiana* showed maximum size of inhibition zone i.e. 14.1mm against *Staphylococcus aureus* and minimum 9.2mm against *E. coli* in aqueous extracts. So far in *Lyngbya maintensiana* there was

not much variations of antibacterial property between aqueous and methanolic extracts was observed. Among the test four species *Phormidium mole* and *Phormidium angustissium* showed moderate antibacterial activity in both aqueous and methanolic extracts which ranges from 3-9mm and 4-12mm diameter size of zone of inhibition in these two species respectively. In other two species *Phormidium bohneri* and *Lyngbya chaetomorphae* the antibacterial property as expressed in zone of inhibition ranges between 3-5.3mm and 3- 6.7mm respectively. However in these four species the antibacterial property was not detected against few pathogenic test species of bacteria in aqueous and methanolic extracts as mentioned in the table-1.

The results so far obtained was encouraging as all the strains of maine cyanobacteria isolated from coastal region of Orissa showed antibacterial activity, although the degree of antibacterial activity varies with species. Among the test species of cyanobacteria *Phormidium tenue* and *Lyngbya maintensiana* showed promising antibacterial activity against multiple strains of pathogenic bacteria in varied pattern in the aqueous and methanolic extracts. This

variation might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extracts as observed by Sastry and Rao, (1994). The antibacterial activity was found to be higher in aqueous extracts than the methanolic extracts, this is probably because of polar nature of active compounds. The differential antibacterial response of the test cyanobacteria to Gram positive and Gram negative group of bacteria may be attributed to the production of active compounds. The result of this work indicates that's the marine cyanobacteria of Orissa coast which displays potential source of bioactive compounds which can be biotechnologically exploited for industrial application. Orissa coast extends over 480km along Bay of Bengal from Chandipur in the north to Gopalpur in the south with Puri coast at the centre. The samples were collected in these regions which are quite apart in their physiographic and environmental conditions and harbours different cyanobacterial strins suitable for growth in such habitats which needs further periodical study at different locations along the coastal region in this direction to reach at a conclusion.

Table 1. Antibacterial activity of Cyanobacterial extracts against Gram positive and Gram negative bacteria as presented by inhibition zone diameter (in mm).

Cyanobacterial strains	Solvent used for extraction	Gram positive bacteria				Gram negative bacteria		
		<i>Staphylococcus epidermidis</i>	<i>Staphylococcus aureus</i>	<i>Bacillus brevis</i>	<i>Bacillus subtilis</i>	<i>Streptococcus aureus</i>	<i>Escherichia coli</i>	<i>Shigella flexinina</i>
<i>Phormidium tenue</i>	Aqueous	18.8	14	9	9	10	17.6	10.5
(Menegh. Gomon) <i>Lyngbya maintensiana</i>	Methanol	9.8	10	8.2	15	9.8	8	8.3
	Aqueous	13.1	14.1	10.5	10	11.1	9.2	11.5
(Menegh)Gomon <i>Phormidium mole</i>	Methanol	10.1	11.1	9.8	9.3	8.8	9	9
	Aqueous	9	5	4	5	5.5	ND	3
(Kutz. Gomon)	Methanol	4	ND	3	ND	ND	ND	4
<i>Phormidium angustissium</i> (W. et G.S. West)	Aqueous	4.8	4	12	3.7	ND	ND	8.9
	Methanol	4	ND	9.8	4	ND	ND	5.4
<i>Phormidium bohneri</i> (Schmidle)	Aqueous	3	4.9	ND	4	3	3.7	4
	Methanol	4	4.1	ND	4	ND	5.3	3.5
<i>Lyngbya chaetomorphae</i> (Iyengar et Desikachary)	Aqueous	5	6.7	4.0	4.5	3	4.7	4.5
	Methanol	3.8	3.9	ND	ND	ND	4.1	4

CONCLUSION

Results from the present work indicate that the species of marine cyanobacteria from orissa coast examined showed a variety of antimicrobial activities and presence of bioactive molecules. Further isolation and identification of the active ingredients need to be done in order to understand their

bioprospects. Since different activities were observed in extracts obtained with organic solvent and extracts obtained with water we can suggest that compounds with different polarities are involved. Thus the present work will contribute to an understanding that these bioactive compounds will need further studies to identify the chemical

structure of these compounds and to examine their beneficial effect for inhibition of some pathogenic bacteria as antimicrobial metabolites of marine cyanobacteria are of special interest now in the development of new environment harmless.

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