EFFECT OF LOW LEVEL LASER IRRADIATION ON NERVE CONDUCTION VELOCITY OF EXPERIMENTALLY INDUCED DIABETIC NEUROPATHY IN WISTAR RATS.

Synopsis Submitted to

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INTRODUCTION

Diabetes mellitus is a metabolic disorder and is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Atkinson and Maclaren, 1994).Diabetes is one of the most common and serious problem in our country which affects all the systems of the body among which its impact over peripheral nerve is severe leading to diabetic neuropathy which produces neuropathy pain, sensory loss, weakness and functional deficit.

Peripheral nerve dysfunction is a common complication of human diabetes mellitus (Clements1979, Sidenius 1982).Clinical symptoms of peripheral neuropathy are present in approximately 25% of diabetic individuals, while nearly all diabetics have a reduction of nerve conduction velocity (spritz ,campbell 1978).

Peripheral nerve pathology in diabetic polyneuropathy is characterized by axonal atrophy and degeneration. The development of the most common form of diabetic neuropathy is symmetrical polyneuropathy, is thought to be conditioned by some chronic metabolic disturbance, for recent pathological studies seem to exclude occlusive vascular disease as a primary causative factor. However, the importance of insulin deficiency in the pathogenesis of diabetic polyneuropathy is still disputed because of ambiguities in the data concerning the relationship between the degree of antecedent 'diabetic control" and the development of this syndrome and the response to insulin treatment in patients with polyneuropathy.

Patients with diabetic polyneuropathy exhibit decreased peripheral motor and sensory nerve conduction velocities, and similar alterations have been found in long-standing diabetics who have no evidence of polyneuropathy. In both of these instances, the decreased nerve conduction velocities are associated with lesions in peripheral nerve biopies, which are more marked in the patients with polyneuropathy.These lesions include loss of myelinated axons, evidence of segmental demyelination and remyelination, and in some instances Schwann cell proliferation.

Recently it has been reported that newly diagnosed juvenile diabetics frequently have decreased Peripheral motor and sensory nerve conduction velocities, despite a normal neurological examination and the absence of symptoms. The pathological lesions, if any, present in the peripheral nerves of newly diagnosed juvenile diabetics have not been identified. The initiation of insulin treatment appears to result in improvement in peripheral nerve conduction velocity in juvenile diabetics ,however, there is evidence that nerve conduction velocity tends to decrease with increasing duration of disease in a manner that cannot be attributed to the effects of age There is, as yet, no clear evidence that the progression of impaired nerve conduction velocity in juvenile diabetics correlates with the progression of the lesions found in association with overt polyneuropathy.

Scientific study of Neuroregenerative effects of low level laser irradiarion on experimentally induced diabetic wistar rats

In 1964 Eliasson found that the induction of experimental diabetes in rats by pancreatectomy or alloxan administration resulted in impaired sciatic motor and sensory nerve conduction velocities within 2 week. This impairment did not occur in rats that failed to become hyperglycemic after exposure to Alloxan. However, Eliasson was unable to prevent the development of impaired nerve conduction velocities by insulin treatment, or to affect it by the addition of insulin to the isolated nerve in vitro. Although there have been two reports that impaired nerve conduction velocity in rats with experimental diabetes can be improved by insulin treatment it has not been possible to prevent its development, and Sharma and Thomas concluded that "the influence of insulin on conduction velocity in diabetic animals is so far uncertain" .

Rockind (1987) studied using direct 632.8nm HeNe laser irradiation to determine the effect of focused laser beams on aggregates of rat fetal brain cells and rat adult brain. The direct He-Ne laser irradiation 3.6J/cm2 caused a significant amount of sprouting of cellular processes outgrowth in aggregates, compared to small amounts produced by non irradiated controls. This observation suggests that low power laser irradiation applied to

the area of an experimentally injured nerve may induce axonal processes sprouting, thereby improving nerve tissue recovery. The mechanism of low power laser on nerve tissue is not completely understood, but some studies partially explain the photochemical effect of laser irradiation on the biological system. The Cytochromes are affected, thereby stimulating Redox activity in the cellular respiratory chain, thereby causing increases in ATP production which activates Na+, K+ -ATPase and other ion carriers, thereby increasing cell activation.

Anders et al (1975) studied on neuro regenerative and neuro protective effects of low level laser and concluded that there is massive axonal sprouting and increase in various molecules such as growth associated protein – 43 (GAP- 43), calcitonin gene related (CGRP) and transforming growth factors betal. They concluded that laser irradiation stimulates the proliferation of the Schwann cells which are key factors for successful nerve recovery.

The present study focuses on the Neuro regenerative effect of therapeutic low level LASER on diabetic neuropathy. LASER as a therapeutic modality in the field of physiotherapy has undergone various researches since early 1960's and many therapeutically significant results have been obtained from LASER.

NEED FOR THE STUDY

The extensive review of literature reveals that there is paucity of studies on role of neuroregenerative effect of low level laser therapy in experimentally induced diabetic neuropathy in wistar rats. Diabetic neuropathy is a major complication mainly due to its severity in symptoms. This particular problem affects large population in our country which needs to be addressed efficiently. Diabetic neuropathy produces various symptoms like pain, sensory loss, weakness etc, particularly lower limb is mostly affected which is primary for locomotion and for ADL so this particular problem has to be addressed effectively and conservatively to provide relief to persons affected by this problem. The scope of management is also very less for this particular complication, this urges to do a

study on this problem with laser which is recognized worldwide for its tissue healing properties.

Current Status both Internationaly And Nationaly

International status

Developed countries with their modern setup "know how" progressing rapidly in exploring the possibilities of low level laser to supplement the medical management and facilitate early recovery.

National status

The wealth of knowledge that has been passed by our researchers regarding the use of low level laser therapy for diabetic neuropathy has to be collaborated with detailed well organized study. Such a scientific study will facilitate indian researchers and clinicians to use this modalities effectively to treat diabetic neuropathy.

OBJECTIVES

1. To find out the effect of various dosage of low level laser therapy on motor nerve conduction velocity (MNCV) of experimentally induced diabetic neuropathy.

2. To find out the effect of various dosage of low level laser therapy on sensory nerve conduction velocity (SNCV) of experimentally induced diabetic neuropathy.

Ethical Committee Clearance Number: IAEC. NO. BPT/001/2008

MATERIALS & METHODS

Materials

1. Laser Unit – Physitalia (Unilaser Scan – 2000)

2. Nerve Conduction Velocity Unit – BIOTECK-NEUROCARE

Study Design: Experimental study

Sampling: simple Random Sampling

Study Centre

Biomedical Research Unit & Laboratory Animal Centre (BRULAC) – (CPCSEA Approved), Saveetha University

RESEARCH LAB - College of Physiotherapy, Saveetha University

Inclusion

Healthy adult male Albino wistar rats in house–bred in polypropylene cages with paddy husk bedding. Standard rat pellete food & tap water was provided and hygienic environment was maintained by cleaning cages twice a week and all rats were housed as 3 in each cage

Species: Rattus norvegicus

Age : 2-3months

Weight: 180-200gms

Sex : Male

STUDY PERIOD: 3 months

PROCEDURE

The experimental rats were on fasting for 24 hours prior to experimentation and were rendered diabetic by a single dose of intra- peritoneal injection of Alloxan 150 mg/kg body weight by dissolving in normal saline (Vogel and Gang, 2002). Diabetes was confirmed with help of one touch ultra glucometer readings by obtaining blood samples from tail end and rats with blood sugar level more than 200 mg/dl were selected were divided into six in each group for further intervention.

Diabetic levels and the weight of the animals were monitored prior to induction of alloxan and regularly in 24hrs, 15, 30, 60 days respectivevely post alloxan induction. On the day 30 booster dose of alloxan of 50mg/bw was induced intra-peritonealy to maintain the diabetic status.

Nerve Conduction Study: Nerve conduction velocities were recorded initially pre Alloxan administration and 30 days and 60 days post induction. Experimental animals were anesthetized with Ether solution and electrode placement areas were shaved and cleaned with alcohol, MNCV recordings were done by fixing surface stimulating electrode at sciatic notch and the tibial nerve posterior to the medial malleolus. Recording electrode was fixed in the dorsal interosseous space of foot. Supra maximal stimulation (6mA) was given and conduction velocity was calculated by measuring distance between two electrodes. SNCV recordings were done for the sural nerve, the anode was placed on the third toe of the foot, and the cathode was placed on the heel of the foot. The frequency band was inclusive of two, 10 Hz muscle potential recordings (orthodromic, motor), 2-Hz potential recordings (antidromic, sensory).

Laser Therapy: The experimental animals with motor and sensory degeneration which confirmed neuropathic status were grouped and treated with low level He-Ne laser therapy of 632.8nm at the site of sciatic notch where the nerve is superficial with various dosage of laser ranging from 3-8j/cm2. The effect of laser induced nerve regeneration was again measured with NCV to find regeneration

In each Laser group the dosage was calculated using following formula:

$$D = p \times t/A$$

D = Dose measured in joules per square centimeters

P= Laser output in milli-watt and it needs to be converted into Watts. In our equipment it has 10 mw output (divided by 1000 to convert to Watts) = 0.01W

t= time in seconds

A= Area of the irradiation site measured in centimeters square

Groups:

I Group- 3 j/cm2 of HE-ne laser irradiation

II Group- 4 j/cm2 of He-ne laser irradiation

III Group- 5 j/cm2 of He-ne laser irradiation

IV Group- 6 j/cm2 of He-ne laser irradiation

V Group- 7 j/cm2 of He-ne laser irradiation

VI Group- 8 j/cm2 of He-ne laser irradiation.

VII Group is kept as control with no laser irradiation

OUTCOME MEASURES

Blood glucose values measured from one touch ultra Glucometer.

MNCV & SNCV values measured from EMG-NCV from BIOTECK

STATISTICAL DATA ANALYSIS

Paired‘t’ test analysis was made for comparison within the groups &

Un-paired t test analysis was made for comparison between the groups.

Table 1: Mean Blood glucose levels at pre Alloxan(Day 0) administration and various days post Alloxan Administration

Nos.	Day 0 (mg/dl)	Day 1 (mg/dl)	Day 15 (mg/dl)	Day 30 (mg/dl)	Day 60 (mg/dl)
42	82.42	261.71	342 .07	389.14	401.42

Table 2: Comparison of Mean Blood Glucose pre & post Alloxan administration using paired t test

S.no	N	Blood Glucose comparison	Pre Alloxan (mg/dl)		Post Alloxan (mg/dl)		T value	P value
			Mean	SD	Mean	SD		
1.	42	Day0 vs Day1	82.4	3.46	261.74	3.49	264.54	<0.0001
2.	42	Day0 vs Day15	82.4	3.46	342.07	3.74	419.64	<0.0001
3.	42	Day0 vs Day30	82.4	3.46	389.14	3.87	397.36	<0.0001
4.	42	Day0 vs Day60	82.4	3.46	401.42	14	66.37	<0.0001

Table 3 : MNCV Values in m/sec of Experimentally Diabetes Induced Wistar Rats

Nos.	Pre test (m/sec)	Post test (30 days) (m/sec)	Post test (60 days) (m/sec)
42	51.71	46.9	33.19

Table 4: Comparison of MNCV values (m/sec) between day 0 (PreAlloxan) to Day 30 & Day 60 (Post Alloxan) with paired‘t’ test

S.no	N	Comparison	Pre Alloxan (m/sec)		Post Alloxan (m/sec)		T value	P value
			Mean	SD	Mean	SD		
1	42	Day 0 vs Day 30	51.7	2.56	46.90	1.220	10.637	<0.0001
2	42	Day 0 vs Day 60	51.7	2.56	33.19	2.682	12.195	<0.0001

Table 5: Sensory Nerve Conduction Velocity Values (m/sec) of Experimentally Diabetes Induced Wistar Rats

Nos	Pre test (m/sec)	Post test (30 days) (m/sec)	Post test (60 days) (m/sec)
42	54.2	47.4	38.56

Table 6: Comparison of SNCV values between day 0 (Pre Alloxan) and day 60 (Post Alloxan) with paired ‘t’ test

s.no	Comparison	Nos.	Pre Alloxan (m/sec)		Post Alloxan (m/sec)		T value	P value
			Mean	SD	Mean	SD		
1	Day0 vs Day 30	42	54.2	2.19	47.4	1.35	20.045	<0.0001
2	Day0 vs Day 60	42	54.2	2.19	38.5	2.25	14.145	<0.0001

Table 7: MNCV values Pre & Post Laser irradiation

s.no	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	pre	post	pre	post	pre	post	Pre	post	pre	post	pre	post	pre	post
1	32.4	42.1	28.4	48.3	35.6	40.2	34.7	38.2	34.5	36.2	36.4	33.9	33.6	35.1
2	30.4	44.6	29.3	49.6	32.3	44.3	35.7	37.8	35.6	37.5	29.4	34.5	36.4	33.2
3	35.3	45.3	30.4	50.1	31.6	38.4	33.2	39.3	34.3	35.4	36.7	32.6	29.4	35.6
4	30.6	43.8	29.4	48.4	29.4	42.3	32.1	40.4	35.2	33.9	35.4	35.1	36.7	34.2
5	31.2	45.4	30.6	48.7	34.3	39.6	29.4	41.8	35.6	33.9	34.3	33.2	35.4	36.7
6	34.3	45.1	30.7	49.9	33.6	41.2	33.2	40.7	33.6	35.2	35.6	36.1	34.3	34.9
mean	**32.3**	**44.3**	**29.8**	**49.1**	**32.8**	**41**	**33.1**	**39.7**	**34.8**	**35.3**	**34.6**	**34.2**	**34.3**	**34.9**

Table8: Comparison of MNCV values (m/sec) prior and 30 days after laser irradiation within the groups

Groups (n=6)	Pre laser irradiation (m/sec)		Post laser irradiation (m/sec)		T value	P value
	Mean	SD	Mean	SD		
Group I 3j/cm2	32.3	2.034	44.3	1.264	14.08	<0.0001 Extremely significant
Group II 4j/cm2	29.8	0.914	49.1	0.794	60.9	<0.0001 Extremely significant

Group III 5j/cm2	32.8	2.190	41	2.097	5.7877	0.0022 Very significant
Group IV 6j/cm2	33.1	2.192	39.7	1.544	4.4355	0.0068 Very significant
Group V 7j/cm2	34.8	0.802	35.3	1.38	0.8344	0.4421 Not significant
Group VI 8j/cm2	34.6	2.699	34.2	1.277	0.3109	0.7684 Not significant
Group VII control	34.3	2.679	34.9	1.195	0.4728	0.6563 Not significant

	Sum of Squares	df	Mean Square	Fisher F-value	Significance (*p*)
Between Groups:	**1,102.851**	**6**	**183.809**	**92.084**	**0.000**
Within Groups:	**69.863**	**35**	**1.996**		
Total:	**1,172.715**	**41**			

Table 9: Post hoc Analysis for MNCV values comparison between the groups

Comparison	Mean 1	Mean 2	N1	N2
1: group 1	+ 32.3	+ 44.5	6	6
2: group 2	+ 29.8	+ 49.1	6	6
3: group 3	+ 32.8	+ 41.0	6	6
4: group 4	+ 33.1	+ 39.7	6	6
5: group 5	+ 34.8	+ 35.3	6	6
6: group 6	+ 34.6	+ 34.2	6	6
7: group 7	+ 34.3	+ 34.9	6	6

Mean Square= 1.996 DF= 35

Confidence intervals

Comparison	Mean1 - Mean2	95% CI of difference
1: group 1	- 12.2	- 14.5 to - 9.9
2: group 2	- 19.3	- 21.6 to - 17.0
3: group 3	- 8.2	- 10.5 to - 5.9
4: group 4	- 6.6	- 8.9 to - 4.3
5: group 5	- 0.5	- 2.8 to + 1.8
6: group 6	+ 0.4	- 1.9 to + 2.7
7: group 7	- 0.6	- 2.9 to + 1.7

Statistical Significance

Comparison	Significant? (P <0.05?)	t
1: group 1	<0.0001-Yes	14.957
2: group 2	<0.0001-Yes	23.661
3: group 3	0.0002-Yes	10.053
4: group 4	0.0005-Yes	8.091
5: group 5	0.5667-No	0.613
6: group 6	0.6449-No	0.490
7: group 7	0.4948-No	0.736

Table 10: SNCV Values Pre & Post Laser Irradiation

s.no	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	pre	post	pre	post	pre	post	Pre	post	pre	post	pre	post	pre	post
1	36.2	46.3	39.3	46.3	41.2	40.2	38	42.1	40.1	39.4	39.4	36.6	38.1	34.4
2	35.3	44.2	37.4	48.2	40.3	42.4	37.2	40.3	37.8	36.3	40.1	35.7	37.1	32.9
3	39.3	45.5	33.6	46.8	41.4	44.6	40.3	39.1	39.2	39.8	37.6	38.1	36.4	36.4
4	39.7	45.7	35.7	51.4	41.2	40.4	39.3	38.6	38	40.4	37	38.2	38.4	34.9
5	38.6	46.9	39.4	47.3	39.7	38.9	36.9	42.4	40.2	41.1	37.3	37.5	37.2	35.7
6	38.4	43.2	40.4	49.6	38.6	41.1	41.7	41.6	38.3	38.2	39.5	37.1	39.1	36.1
Mean	**37.9**	**45.3**	**37.6**	**48.2**	**40.4**	**41.2**	**38.9**	**40.6**	**38.9**	**39.2**	**38.4**	**37.2**	**37.7**	**35.06**

Table 11: Comparison of SNCV values (m/sec) within the groups prior and 30 days after laser irradiation.

Groups(n=6)	Pre laser irradiation (m/sec)		Post laser irradiation (m/sec)		T value	P value
	Mean	SD	Mean	SD		
Group I -3j/cm2	37.9	1.766	45.3	1.370	8.9299	0.0003 Extremely significant
Group II- 4j/cm2	37.6	2.593	48.2	1.926	7.8523	0.0005 Extremely significant
Group III- 5j/cm2	40.4	1.097	41.2	1.996	1.0985	0.3220 Not significant
Group IV- 6j/cm2	38.9	1.879	40.6	1.599	1.5536	0.1810 Not significant
Group V- 7j/cm2	38.9	1.058	39.2	1.724	0.4802	0.6514 Not significant
Group VI- 8j/cm2	38.4	1.332	37.2	0.951	1.4112	0.2173 significant
Group VII- control	37.7	0.991	35.06	1.297	4.0737	0.0096 significant

Results of One way Anova:

	Sum of Squares	df	Mean Square	Fisher F-value	Significance (p)
Between Groups:	**740.912**	**6**	**123.485**	**48.875**	**0.000**
Within Groups:	**88.430**	**35**	**2.527**		
Total:	**829.342**	**41**			

Table 12: Post hoc Analysis for SNCV values comparison between the groups

Comparison	Mean 1	Mean 2	N1	N2
1: group1	+ 37.9	+ 45.3	6	6
2: group 2	+ 37.6	+ 48.2	6	6
3: group 3	+ 40.4	+ 41.2	6	6
4: group 4	+ 38.9	+ 40.6	6	6
5: group 5	+ 38.9	+ 39.2	6	6
6: group 6	+ 38.4	+ 37.2	6	6
7: group 7	+ 37.7	+ 35.6	6	6

Mean Square= 2.527 DF= 35

Confidence intervals

Comparison	Mean1 - Mean2	95% CI of difference
1: group1	- 7.4	- 10.0 to - 4.8
2: group 2	- 10.6	- 13.2 to - 8.0
3: group 3	- 0.8	- 3.4 to + 1.8
4: group 4	- 1.7	- 4.3 to + 0.9
5: group 5	- 0.3	- 2.9 to + 2.3
6: group 6	+ 1.2	- 1.4 to + 3.8
7: group 7	+ 2.1	- 0.5 to + 4.7

Statistical Significance

Comparison	Significant? (P <0.05?)	t
1: group1	0.0005-Yes	8.063
2: group 2	<0.0001-Yes	11.550
3: group 3	0.4231-No	0.872
4: group 4	0.1232-No	1.852
5: group 5	0.7569-No	0.327
6: group 6	0.2481-No	1.307
7: group 7	2.288- No	2.288

RESULT ANALYSIS

Blood glucose: Table 1 Blood glucose levels were initially noted on the day 0 with M= 82.42 , on day1 after alloxan induction as M=261.71,on day 15 as M=342.07, on day 30 as M=389.14 and 0n day 60 as M=401.42 and statistical analysis were made comparing the mean and standard deviation values of day 0 with day1,day15,day30 & day 60 and result obtained showed significant changes in blood glucose levels for all t values showing p value <0.0001 as given in Table 2.

MNCV recording for confirming motor degeneration: Table 3 MNCV results of tibial branch of sciatic nerve in all the animals were recorded on day 0 prior alloxan induction with M=51.71 and on day 30 after alloxan induction which showed M=46.9 and on 60^{th} day which confirmed M=33.19 and statistical analysis were made with values of day 0 with day30 & day 60 which showed significant changes in MNCV with p value <0.0001 of t values10.637 &12.195 as shown in Table 4.

SNCV recording for confirming sensory degeneration: Table 5 SNCV results of Sural nerve in all the animals were recorded on day 0 prior alloxan induction with M=54.2 and on day 30 after alloxan induction which showed M=47.4 and on 60^{th} day which confirmed M=38.56 and statistical analysis were made with values of day 0 and day 60 which showed significant changes in SNCV with p value <0.0001 of t value20.045 & 14.145 as shown in table 6.

MNCV recording for confirming neuro regeneration: Results shown in Table 7 were statistically analyzed with comparing the pre and post laser irradiation among all the 7 groups and the results showed that Group I with 3j/cm2 showed extremely significant changes with p value <0.0001 of t value=14.08 and Group II with 4j/cm2 also showed extremely significant changes with p value <0.0001 of t value=60.9 and Group III with 5j/cm2 showed very significant changes with p value=0.0022 of t value=5.787 and Group IV with 6j/cm2 also showed very significant changes with p value =0.0068 of t value=4.435 and Group V with 7j/cm2 showed no significant changes with p value

=0.4421 of t value=0.834 and Group VI with 8j/cm2 showed non significant changes with p value =0.768 of t value=0.3109 and Group VII which were kept as control without laser irradiation also showed statistically non significant effect with pvalue0.6563 of t value=0.4728 as shown in Table 8.

Comparison of MNCV values between the groups after laser irradiation using one way ANOVA & Post Hoc test.

Analysis of variance of MNCV values Post laser irradiation revealed significant p value <0.0001 with F – value 92.084 and post hoc analysis revealed that groups 1,2,3,4 showed significant improvement and groups 5,6,7 did not show statistical significance for 95% confidence interval as shown in table 9.

SNCV recording for confirming Neuro regeneration: Results shown in Table 10 were statistically analyzed with paired t test comparing the pre and post laser irradiation among all the 7 groups and the results showed that Group I with 3j/cm2 showed extremely statistically significant changes with p value =0.0003 of t value= 8.9299 and Group II with 4j/cm2 also showed extremely statistically significant changes with p value =0.0005 of t value=7.8523 and Group III with 5j/cm2 showed No significant changes with p value=0.3220 of t value=1.0985 and Group IV with 6j/cm2 also no significant changes with p value = 0.1810 of t value=1.5536 and Group V with 7j/cm2 showed non-significant changes with p value =0.6514 of t value=0.4802 and Group VI with 8j/cm2 showed non significant changes with p value =0.2173 of t value=1.4112 and Group VII which were kept as control without laser irradiation also showed statistically non significant effect with p value=0.0096 of t value=4.0737 as shown in table 11.

Comparison of SNCV values between the groups after laser irradiation using one way ANOVA & Post Hoc test.

Analysis of variance of SNCV values Post laser irradiation revealed significant p value <0.0001 with F – value 48.875 and post hoc analysis revealed that groups 1,2 showed

significant improvement and groups 3,4,5,6,7 did not show statistical significance for 95% confidence interval as shown in table 12.

DISCUSSION:

In the present study comparison was done on various dosage of low level laser therapy to find out its neuroregenerative effect in experimentally induced diabetic neuropathy in wistar rats. The diabetes status was confirmed by repeated measures of blood glucose analysis from day 0, day1, day15, day30 and day60. The dosage to induce diabetes was selected as 150mg/ kg b.w of alloxan intraperitonealy based on T.Szkudelski in 2001.

Sven G. Eliasson 1964 & Dharmesh kumar 2008 in their study considered 200mg/dl as base line value for their study and Linda.K.Butler 1995 stated that around 90 mg/dl of blood is normal blood glucose levels for 15-24 hrs fasted rats and they also stated that in diabetic induced rats it may go up to 200-400mg/dl in 3-4 weeks after diabetic induction . As per the earlier studies by Thierry C. Coste2007 and Dharmeshkumar D. 2008, confirmed that diabetic neuropathy will start by 15 days of uncontrolled diabetes and PK Thomas 1981 recorded neuropathic changes after 8 weeks and Greet –Jan Biessels 1999 confirmed this by proving that impairments of sciatic nerve conduction velocities developed fully during the first 2-3 months of diabetes J. G. R. Jefferys 1979 proved in his experiment that normal nerve conduction velocity of wistar rats was around 52m/sec ranged from 46 m/sec to 57m/sec .

Ander et al (1975) underwent study on Neuro regenerative and Neuro protective effects of low level laser and concluded that there is massive axonal sprouting and increase in various molecules such as growth associated protein – 43 (GAP- 43), calcitonin gene related (CGRP) and transforming growth factors betal. They concluded that laser irradiation stimulates the proliferation of the Schwann cells which are key factors for successful nerve recovery. In the present study also it is confirmed nerve regeneration through nerve conduction velocity studies. Once when the neuropathy

changes were confirmed the animals which were grouped as 7 were administered with different dosage of laser ranging from 3-8j/cm2 and 1 group were kept as control without laser irradiation.

The MNCV result analysis within the groups showed that laser dosage of 3 & 4,5,6 j/cm2 is having more motor regenerative effect as compared with higher dosage and control group which did not show significant effect and on analyzing experimental group MNCV values between groups showed dosages of 3-6j/cm2 showed significant p values and 7-8j/cm2 and control group did not showed any significant effect.

The SNCV result analysis within the groups showed that laser dosage of 3,4j/cm2 is having more sensory regenerative effect as compared with 5-8j/cm2 dose and control group also did not show significant effect. This MNCV and SNCV results are important finding of the study that the calculation of correct dosage of laser is very important. If dosage not calculated properly it can inhibit the nerve regeneration process like higher dosage can have photo inhibitory effect.

CONCLUSION

In the present study on analyzing the results it can be concluded that low level laser of 3&4j/cm2 is found to be effective in regeneration of both MNCV & SNCV of experimentally induced diabetic neuropathy as compared with control group and with dosage of higher energy with 5-8j/cm2. The present investigation highlights the possible utility of Helium-Neon laser with appropriate energy density as an adjunctive modality for diabetic neuropathy in clinical practice.

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