CHAPTER 4
METHODOLOGY

4.1. MATERIALS & METHODS

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

Materials

1. Laser Unit – Physitalia   (Unilaser Scan – 2000), Class-1, Type-B, 230V-50Hz
2. Nerve Conduction Velocity Unit – BIOTECK-NEUROCARE
3. Glucometer: one touch ultra (Johnson & Johnson, USA)

4.2. STUDY DESIGN:   RCT

Sampling: Simple Random Sampling

Study Centre

Biomedical Research Unit & Laboratory Animal Centre (BRULAC) - CPCSEA
Approved, Saveetha University, Chennai.

RESEARCH LAB - College of Physiotherapy, Saveetha University

4.3. INCLUSION

Species: Rattus norvegicus
Age      : 2-3months
Weight: 180-200gms
Sex       : Male
Study Duration: 3 months
Total number of 42 experimental rats was selected for the study based on the inclusion criteria and was kept on fasting for 12 hours prior to experimentation and was 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). Alloxan induction procedure was performed by the investigator under the supervision of veterinarian surgeon at saveetha university animal laboratory.
Blood glucose level of all the rats were measured prior to Alloxan induction and after 24hrs post induction and all the rats were screened for blood glucose levels and rats with blood glucose levels of more than 200 mg/dl were selected for further intervention. Diabetes was confirmed with help of Glucometer readings by obtaining blood samples from tail vein of the rat. Diabetic levels of the rats were monitored prior to Alloxan induction and on day 1, 15, 30 and 60 after Alloxan induction. Booster dose of 50 mg/kg body weight of Alloxan was administered on the 30th day to maintain diabetic status.

**Nerve Conduction Study:**

Nerve conduction velocities for all the rats (motor nerve conduction & sensory nerve conduction) during the full course of study were recorded by an EMG-NCV technician who was blinded about the outcome and interventional procedures. Nerve conduction velocities for all the ungrouped rats 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 Testing:**

MNCV recordings were done by fixing 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 Testing:**

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).

Results of both MNCV and SNCV tests pre and post alloxan induction were tabulated and analyzed statistically to confirm the neuropathic status.

**Phase II:**

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Phase II          GROUPING
                  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

LASER irradiation
for 4 sessions in
a week for 4 weeks

MNCV & SNCV recording to analyze effect of LASER irradiation
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After analyzing motor and sensory nerve conduction velocity tests values, the neuropathic status was confirmed and the rats were randomized into study groups and control group for phase II of experimentation.

The experimental animals with motor and sensory degeneration which confirmed neuropathic status were randomized into seven groups (six rats in each group) and irradiated with low level laser therapy with various dosages as follows.

**Groups:**

I Group - 3 j/cm\(^2\) of He-Ne laser irradiation  
II Group - 4 j/cm\(^2\) of He-Ne laser irradiation  
III Group - 5 j/cm\(^2\) of He-Ne laser irradiation  
IV Group - 6 j/cm\(^2\) of He-Ne laser irradiation  
V Group - 7 j/cm\(^2\) of He-Ne laser irradiation  
VI Group - 8 j/cm\(^2\) of He-Ne laser irradiation  
VII Group is kept as control

**Laser Therapy:**

The experimental animals under each group were prepared for the interventional procedure by shaving the area and cleaning the part (sciatic notch) to be irradiated with normal saline. Low level He-Ne laser therapy of 632.8nm was irradiated at the site of sciatic notch of the rat where the nerve is superficial and the irradiation was given for 4 days in a week for 4 weeks with various dosage of laser ranging from 3 to 8j/cm\(^2\). The effect of laser induced nerve regeneration was again measured with motor and sensory nerve conduction velocity to find regeneration status of the nerve.

In each Laser group the dosage was calculated using following formula:
D = P x 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

4.5. OUTCOME MEASURES

Blood glucose values measured from one touch ultra Glucometer.

MNCV & SNCV values measured from EMG-NCV from Bioteck-Neurocare