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To Investigate Effect of Claoxylon Indicum Leaves Extract for Diabetic Neuropathic Pain in Rats

Mr. Md. Amin Md. Sajid Dosani[1] and Dr. Abhijit V. Shrirao[2]
Student[1] & Professor[2]
Pataldhamal Wadhwani College of Pharmacy, Yavatmal, Maharashtra, India

Abstract: *Diabetic neuropathic pain is one of the most common chronic complications of diabetes mellitus and is characterized by hyperalgesia, allodynia, and progressive nerve damage associated with oxidative stress and inflammation. Despite the availability of conventional therapies, their limited efficacy and adverse effects necessitate the search for safer and more effective alternatives from medicinal plants. The present study was designed to investigate the effect of Claoxylon indicum leaves extract on diabetic neuropathic pain in rats. Diabetes was induced in experimental animals using streptozotocin (STZ), followed by the development of neuropathic pain symptoms. The animals were divided into different groups, including normal control, diabetic control, standard treatment, and test groups receiving varying doses of Claoxylon indicum leaves extract. Neuropathic pain was assessed using behavioral parameters such as thermal hyperalgesia, cold allodynia, and mechanical nociception. Biochemical estimations including blood glucose level, oxidative stress markers, and antioxidant enzyme activity were also evaluated. Histopathological examination of sciatic nerve tissue was performed to determine neuroprotective effects. The extract demonstrated significant attenuation of neuropathic pain responses, reduction in blood glucose levels, and improvement in antioxidant status when compared with diabetic control animals. Histological studies further supported the protective effect of the extract against nerve degeneration. The findings suggest that Claoxylon indicum leaves extract possesses potential antidiabetic and neuroprotective activities, possibly mediated through antioxidant and anti-inflammatory mechanisms. Therefore, the plant may serve as a promising natural therapeutic candidate for the management of diabetic neuropathic pain.*

Keywords: *Diabetic neuropathic*

I. INTRODUCTION

Diabetes Mellitus (DM), commonly known as just diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. As of 2019, an estimated 463 million people had diabetes worldwide (8.8% of the adult population),with type 2 diabetes making up about 90% of the cases. Rates are similar in women and men. In 2019,diabetes resulted in approximately 4.2 million deaths.(Amir Aslam et al.,2014)

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secrection, insulin action or both (Oliveria H,2008)

The level of hyperglycaemia associated diabetes increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life.(Piero N,2015)

Glucose blood levels are maintained by insulin which is a hormone released from the pancreas. When these level increases, insulin is produced from the pancreas and maintained the level of glucose. In diabetic patients, the production of insulin is absent or less which causes hyperglycemia (Sonia Verma & Madhu Gupta,2018)

A prospective study in Finland followed newly diagnosed diabetes patients between the ages of 45 and 64 years for 10 years. It found a 6% prevalence at the time of diagnosis of diabetes and a 26.4% prevalence at the 10-year follow-up

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(Amir Aslam et al.,2014).In a large UK-based community diabetic population, Abbot observed that increasing age was directly related to painful symptoms of neuropathy. Most studies found no significant difference in gender; however, Abbot et al. reported a slightly higher prevalence of painful symptoms of neuropathy in females (38%) than males (31%). The same study also found a higher prevalence of painful symptoms in South Asians (38%) compared to Europeans (32%)(Amir Aslam et al.,2014).

II. EXPERIMENTAL WORK

MATERIALS

Chemicals & Reagents

SR NO.	NAME OF CHEMICAL	COMPANY
1.	Petroleum Ether	Merk Chemical
2.	Methanol	OAISIS Alcohol India Pvt Ltd.
3.	Chloroform	ACME
4.	Streptozotocin (STZ)	M P Biomedical
5.	Molish Reagent	S D Fine Chem Ltd.
6.	Mayers Reagent	S D Fine Chem Ltd.
7.	Sulphuric acid	S D Fine Chem Ltd.
8.	Lead acetate	ACME
9.	Ferric chloride	S D Fine Chem Ltd.
10.	Sodium hydroxide	ACME
11.	Hydrochloric acid	S D Fine Chem Ltd.
12.	Zinc	S D Fine Chem Ltd.
13.	Citric acid	S D Fine Chem Ltd.
14.	Sodium citrate	S D Fine Chem Ltd.
15.	Glucose diagnostic kit	Ambica Diagnostic

INSTRUMENTS

SR NO.	NAME OF INSTRUMENT	COMPANY/MODEL
1.	Analgesiometer	HICON
2.	Rotarod appratus	HICON
3.	Micro cooling centrifuge	REMI
4.	Uv – visible spectroscopy	Thermo

Procurement and Authentication of plant materials

Claoxylon indicum plant were collected from local region of Maharashtra. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agricultural Biotechnology , Yavatmal.

Experimental Animals

Healthy sprague dawley male rats approximately 8 weeks of age weighing about 200-210 gm were purchased from NIN Hyderabad, India were used for the pharmacological screening. The animals were housed in polypropylene cages with wire mesh top and husk bedding and maintained under standard environmental conditions (22+20C), relative humidity 55-60%,light dark cycle of 12 hours each and fed with standard pellet diet and water. The protocols for all the animal studies were approved by the Institutional Animal Ethical Committee (IAEC),P.Wadhwani college of pharmacy, yavatmal with research project number 650/PO/Re/S/2002/CPCSEA/2026/08

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III. METHODS

Extraction of Claoxylon indicum leaves

Leaves of Claoxylon indicum plant were collected, dried in shade and coarsely powdered. The powdered leaves were subjected to maceration by methanol and thereafter water to get methanolic & aqueous extract respectively.

Phytochemical Screening :

Methanolic and aqueous extract were subjected to phytochemical screening for the detection of various active constituents.

- **Test for Carbohydrates** (Prashant Tiwari et al.,2011) Dissolve 2 gm extract in 5 ml distilled water and filter it.

The filtrates were used to test for the presence of carbohydrates.

Molish test

A few drops of the extract was treated with molish reagent (Alpha – napthol in alcohol) and a drop of concentrated sulphuric acid was added . Purple color was obtained. This confirms the presence of Carbohydates.

- **Test for Alkaloids (Prashant Tiwari et al.,2011) Hager's test**

Filtrate was treated with saturated aqueous solution of picric acid Presence of alkaoids were confirmed by the formation of yellow coloured precipitate.

- **Test for saponin (Prashant Tiwari et al.,2011) Foam test**

Small quantity of the extract was shaken with 2 ml of water.
Persistance of foam produced for 10 min indicated the presence of saponins.

- **Test for tannin (Prashant Tiwari et al.,2011)**

Take 0.5 gm of the dried powdered plant. Boil o.5 gm sample in 20 ml of water in a test tube. Filter the above mixture.Add few drops of 0.1% ferric
chloride. Development of a brownish green or a blue-black colouration indicated the presence of tannins.

- **Test for terpenoids (Prashant Tiwari et al.,2011) Salkowski Test**

Mix 2 ml of chloroform to extract solution carefully added con. H2So4 to form a layer.A reddish brown colouration of the interface indicated the presence of terpenoids.

- **Test for glycosides (Prashant Tiwari et al.,2011) Killer killani test**

To 5o mg of each extracts, 2 ml of glacial acetic acid, 1 ml Fecl3 solution were added, heated and the cooled. This was transferred to a test tube containing 2 ml conc.H2SO4 and observed.

- **Test for steroids (Prashant Tiwari et al.,2011) Salkowski Test**

To 100 mg of each extracts,2 ml of CHCl3,2 ml of conc. H2SO4 was added,mixed thoroughly and both the layers were observed for colour.

- **Test for phenol (Prashant Tiwari et al.,2011) Ferric chloride test**

Treat the extract with 3-4 drops of ferric chloride solution. Formulation of bluish black colour indicated the presence of phenols.

- **Test for Amino acid (Prashant Tiwari et al.,2011) Ninhydrin Test**

Add ninhydrin reagent to the test solution and boiled for few minutes. Formation of blue colour indicated the presence of amino acids.

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• Test for flavonoids (Prashant Tiwari et al.,2011) Ferric chloride test

Add a few drops of ferric chloride solution to the extract solution.Development of intense green colour indicates the presence of flavonoids.

Lead acetate test

Treat the extract with few drops of lead acetate solution. Formation of yellow precipitate indicate presence of flavonoids.

• Test for anthraquinones (Prashant Tiwari et al.,2011)

1 ml of filter added to 10 ml benzene. Filter and add 5 ml ammonia to thefiltrate and shake well. Pinkish colour gives the presence of anthraquinone.

Experimental Procedure Animal Groups:

For this study animals will be divided into following groups (n=6)

- Group-I (Normal Control) = Animals were treated with normalsaline solution
- Group-II (Negative Control) = Diabetes was induced in rats usingSTZ (60 mg/kg)
- Group-III (High dose of MECI) = Diabetic rats were treated withhigh dose (400 mg/kg) of methanolic extract of Claoxylon indicum
- Group-IV (Low dose of MECI) = Diabetic rats were treated withlow dose (200 mg/kg) of methanolic extract of Claoxylon indicum
- Group-V (High dose of WCI) = Diabetic rats were treated withhigh dose(400 mg/kg) of aqueous extract of Claoxylon indicum
- Group-VI (Low dose of WCI) = Diabetic rats were treated withlow dose (200 mg/kg) of aqueous extract of Claoxylon indicum
- Group VII (Standard) = Diabetic rats were treated with standardanti-diabetic drugs Glibenclamide (5 mg/kg).

Induction of Diabetic Neuropathic Pain

Diabetes was induced by intraperitonial injection of STZ (60mg/kg) to overnight fasted rats. Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day of administration withthe help of glucose diagnostic kit (Ambica diagnostics) showing fasting blood glucose levels above 200mg/dl confirmed as diabetic. Diabetic Neuropathic pain was confirmed after 4 weeks of induction of Diabetes byfollowing models:

Models For Diabetic Neuropathic Pain

Thermal Method Tail Flick Model

The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat(D'Amour et al., 1941). Most commonly, an intense light beam is focused on the animal's tail and a timer starts. When the animal flicks its tail, the timer stops and the recorded time (latency) is a measure of the pain threshold(Tzschentke et al.,2007). Alternate methods can be used to apply heat, such as immersion in hot water.

The rat's tail was subject towards heat generated by electrical stimulus. Cut of period was set at max up to 10 sec. After 28th day of diabetic induction rats under Negative group and treatment group showed hyperalgesia characteristic confirming Neuropathic pain (Dominique Sigaudo Roussel et al.,2007).

Mechanical Method Swimming Endurance Test Model

Endurance training is the act of exercising to increase endurance. The term endurance training generally refers to training the aerobic system as opposed to the anaerobic system. The need for endurance in sports is often predicated as the need of cardiovascular and simple muscular endurance, but the issue of endurance is far more complex. Endurance

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can be divided into two categories including: general endurance and specific endurance. It can be shown that endurance in sport is closely tied to the execution of skill and technique. A well conditioned athlete can be defined as, the athlete who executes his or her technique consistently and effectively with the least effort.(Michael Yessis,2008) Key for measuring endurance are heart rate, power in cycling and pace in running (Friel, 2016).

Rats were immersed in water tank (diameter 18 cm,height 40 cm) filled to a depth of 25 cm with water at 250C. On the first experimental day, rats are gently placed in the water for a 15 min period of habituation. On removal from the water, they were placed in a chamber box under a shed for 30 min to dry. The next day,they were replaced in the tank and observed for 15 min. During this period,the total time that spent immobile (i.e., making only the movements necessary to remain afloat) is measured. Removal time was set at max 15 min. After 28th day rats showed decrease in mean swim time (MST) as compared to control group showing development of Stress. (Dominique Sigaudo Roussel et al.,2007)

Rota Rod Model

The rota rod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test measures parameters such as riding time (seconds) endurance some of the functions of the test include evaluating balance, grip strength and motor coordination of the subjects, especially in testing the effect of experimental drugs or after traumatic brain injury(Mouzon et al.,2012)

The animals were subsequently tested for muscle coordination on a Rota rod rotating at 25 rpm and the duration of stay on the rod was recorded. The mean falling time of Rats was noted after 28th day resulted into decreased in falling time as compared to Control group showing loss of muscle strength. (Dominique Sigaudo Roussel et al.,2007)

Statistical Analysis

The data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnets comparison test with equal sample size and student t-test was compared with unpaired groups. The difference was considered significant when P- values < 0.05*.All the values were expressed as mean + standard deviation (S.D).

IV. RESULTS

The preliminary phytochemical screening of plant leaves extract was carriedout by suitable tests.

Table : Results of Phytochemical Screening of Methanolic and Aqueous Extract of Claoxylon indicum plant.

Sr. No.	Plant Constituents	Test Performed and Reagents	*CI* Methanolic Extract	*CI* Aqueous Extract
1.	Test for Alkaloids	Hager's Reagent	+	+
2.	Test for Carbohydrates	Molisch Test	+	+
3.	Test for Glycosides	Killer Killani Test	+	+
4.	Test for Amino acid	Ninhydrin Test	_	_
5.	Test for Tannins	Ferric Chloride Test Lead Acetate Test	+ +	+ _
6.	Test for Steroids	Salkowski Test	+	+
7.	Test for Phenols	Ferric Chloride Test	+	+
8.	Test for Flavonoids	Ferric Chloride Test Lead Acetate Test	+ +	+ _
9.	Test for Terpenoids	Salkowski Test	+	+
10.	Test for Saponins	Foam Test	+	+
11.	Test for Anthraquinone		_	_

(- Absent) ,(+ present)

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Table : Effect of STZ on blood Glucose level of Rats on 3rd,28th and 42nd day

GROUPS	On 3rd day Glucose level (mg/dl)	On 28th day Glucoselevel (mg/dl)	On 42nd day Glucose level(mg/dl)
Group- I	96.58 ± 2.85	98.07 ± 1.76	96.21 ± 2.71
Group- II	261.85 ± 7.79**	279.03 ± 13.85**	297.73 ± 11.65**
Group- III	260.21 ± 4.25 ns	140.14 ± 5.02**	129.2 ± 5.44**
Group- IV	260.44 ± 6.88 ns	163.57 ± 10.44**	151.91 ± 11.42**
Group- V	261.25 ±3.26 ns	138.58 ± 4.55**	126.01 ± 1.79**
Group- VI	260.85 ± 6.26 ns	164.04 ± 14.64**	155.75 ± 12.11**
Group- VII	261.25 ± 2.25 ns	99.06 ± 2.29**	101.42 ± 3.39**

The results were expressed as Mean ± SD (n = 6),
ns p>0.05, ** p<0.01, *p<0.05 when compared to STZ treated Negative Control group rats.

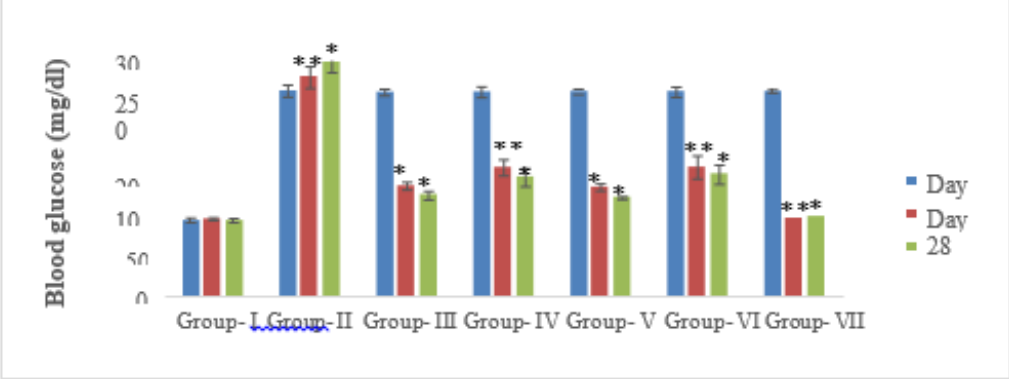

Figure : Effect of STZ on blood Glucose level of Rats on 3rd,28th and 42nd day Table 2 and Figure 3 shows the effect of STZ on blood glucose levels of the rats on day 3rd,28th & 42nd. There was significant increase ($p < 0.01$) in the blood glucose level in negative control group compared to normal control group of rats on 3rd ,28th & 42nd day. Group III (High dose of MECI),Group IV (Low dose of MECI), Group V (High dose of WCI), Group VI (Low dose of WCI) showed significant decrease in ($p < 0.01$) in the Blood Glucose Level compared to Negative control Group.

Table : Effect of STZ on Body weight of Rats on 3rd,28th and 42nd day

GROUPS	Weight of Rats on 3rd day (gm)	Weight of Rats on 28th day (gm)	Weight of Rats on 42nd day (gm)
Group- I	204.67 ± 6.4	206.17 ± 4.53	204.17 ± 3.18
Group- II	197.5 ± 2.73 ns	160.33 ± 3.32**	137.33 ± 1.75**
Group- III	203.5 ± 6.47 ns	198.5 ± 3.08**	203.17 ± 2.48**
Group- IV	201.67 ± 6.71 ns	195.67 ± 1.75**	201.5 ± 1.87**
Group- V	200.67 ± 6.71 ns	197.17 ± 1.94**	200.17 ± 1.47**
Group- VI	199.17 ± 1.72 ns	194.83 ± 3.76**	199.67 ± 1.86**
Group- VII	202.67 ± 6.28 ns	203.5 ± 1.87**	200.26 ± 1.87**

The results were expressed as Mean ± SD (n = 6),

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ns p>0.05, ** p<0.01, *p<0.05 when compared to STZ treated Negative Control group rats.

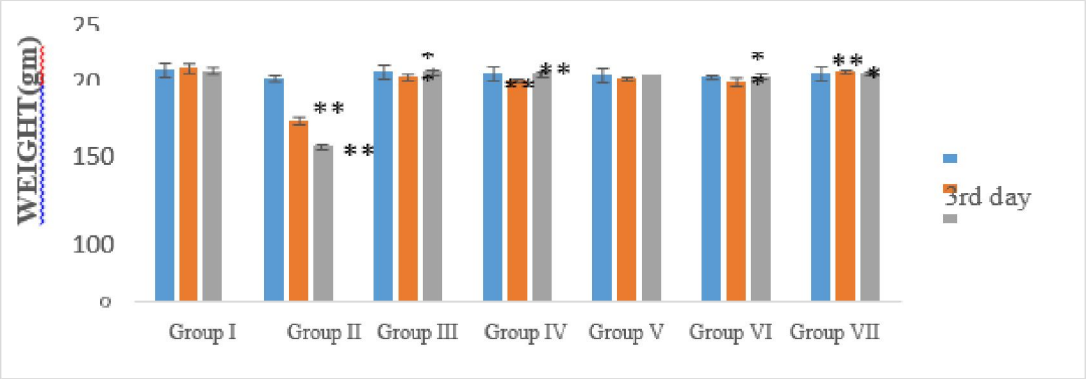

Figure 4 : Effect of STZ on Body weight of Rats on 3rd,28th and 42nd day

Table 3 and Figure 4 shows the effect of STZ on body weight of the rats on day 3rd,28th and 42nd.There was significant decrease ($p < 0.01$) in the body weight in negative control group compared to normal control group of rats on 3rd ,28th & 42nd day. Group III (High dose of MECI),Group IV (Low dose of MECI), Group V (High dose of WCI), Group VI (Low dose of WCI) showed significant increase ($p< 0.01$) in the Body weight compared to Negative control Group.

Table 4 : Tail flick response of Rats on 3rd,28th and 42nd day

GROUPS	On 3rd daytail flick response in(sec)	On 28th day tail flick response in (sec)	On 42nd day tail flick response in (sec)
Group- I	5.42 ± 0.11	5.66 ± 0.03	5.70 ± 0.04
Group- II	5.39 ± 0.01ns	7.36 ± 0.05**	8.29 ± 0.01**
Group- III	5.37 ± 0.06ns	3.20 ± 0.08**	2.15 ± 0.01**
Group- IV	5.38 ± 0.06ns	3.33± 0.03**	2.31 ± 0.03**
Group- V	5.36 ± 0.08ns	3.28 ± 0.04**	2.16 ± 0.01**
Group- VI	5.37 ± 0.06ns	3.39 ± 0.01**	2.27± 0.04**
Group- VII	5.35 ± 0.12ns	3.14 ± 0.02**	2.24 ± 0.02**

The results were expressed as Mean ± SD (n = 6),
ns p>0.05, ** p<0.01, *p<0.05 when compared to STZ treated Negative Control group rats.

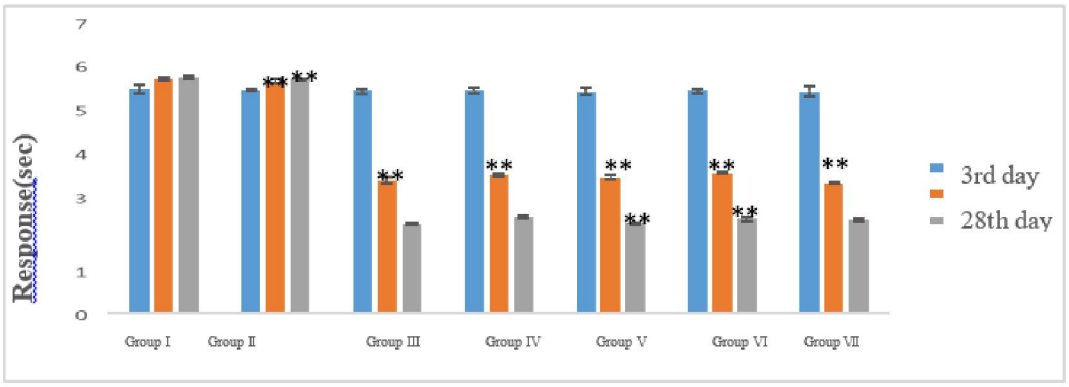

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Figure : Tail flick response of Rats on 3rd,28th and 42nd day Table 4 and Figure 5 reveals the effect of STZ on tail flick pain sensation effect of the rats on 3rd , 28th & 42nd day of STZ induction. There was significant decrease ($p< 0.01$) in the analgesic effect of rats in negative control group compared to normal control group of rats on 3rd,28th & 42nd day. This decrease inthe analgesic effect confirms the hyperalgesia in the rats. Group III (High dose of MECI),Group IV (Low dose of MECI), Group V (High dose of WCI), GroupVI (Low dose of WCI) showed significant increase in ($p < 0.01$) in the Tail flick response compared to Negative control Group.

Table 5 : Muscle Co-ordination response on Rota Rod on 3rd,28th and 42nd day.

GROUPS	On 3rd day mean falling time (sec) MFT	On 28th day mean falling time (sec) MFT	On 42nd day mean falling time (sec) MFT
Group- I	70.50 ± 2.76	74.30 ± 3.08	73.13 ± 1.83
Group- II	65.76 ± 1.59^{ns}	60.86 ± 1.26**	55.87± 2.13**
Group- III	67.71 ± 1.09^{ns}	70.06 ± 1.96**	71.18 ± 2.17**
Group- IV	66.17± 1.71^{ns}	68.82 ± 0.93**	70.24 ± 0.92**
Group- V	68.94± 1.71^{ns}	70.85 ± 1.79**	71.05± 1.74**
Group- VI	68.41 ± 1.65^{ns}	70.15 ± 0.87**	69.98 ± 2.25**
Group- VII	70.70 ± 2.38^{ns}	73.13± 1.75**	72.02 ±1.47**

The results were expressed as Mean ± SD (n = 6),
ns p>0.05, ** p<0.01, *p<0.05 when compared to STZ treated Negative Control group rats.

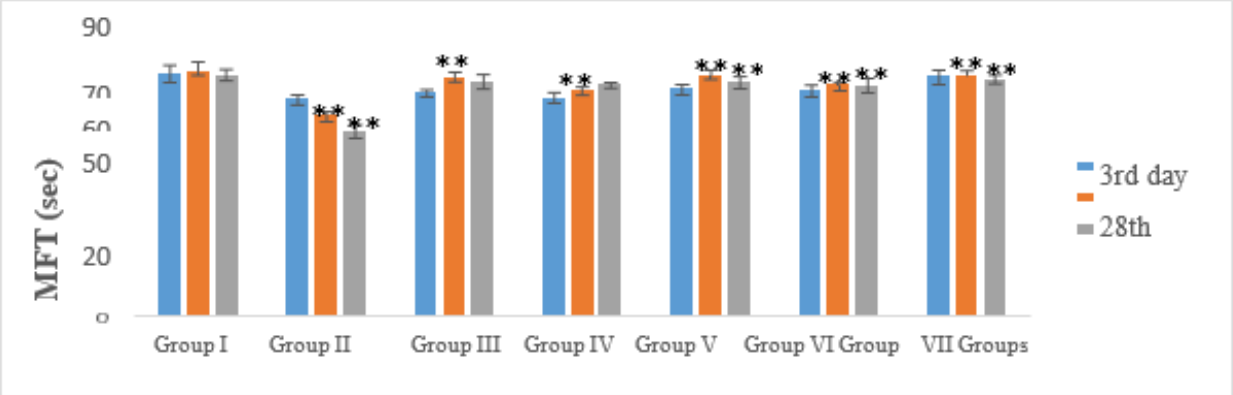

Figure : Muscle Co-ordination response on Rota Rod on 3rd,28th and 42nd day. Table 5 and Figure 6 reflect the effect of STZ on muscle strength of the rats on 3rd, 28th & 42nd day of STZ induction. There was significant decrease ($p < 0.01$) in the muscle strength of rats in negative control group compared to normal control groupof rats on 3rd,28th & 42nd day. This decrease in the muscle coordination confirms the loss of muscle strength in the rats. Group III (High dose of MECI),Group IV (Low dose of MECI), Group V (High dose of WCI), GroupVI (Low dose of WCI) showed significant increase in ($p < 0.01$) in the muscle co-ordinaton compared to Negative control Group.

Table 6 : Swimming Endurance test (SET) on 3rd,28th and 42nd day.

GROUPS	SET On 3rd day in (min)	SET On 28th day in (min)	SET On 42nd day in (min)
Group- I	6.06 ± 0.44	5.47 ± 0.19	4.22± 0.18

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Group- II	5.72 ± 0.10ns	9.34 ± 0.32**	9.28± 0.36**
Group- III	5.60 ± 0.17ns	4.45 ± 0.17**	3.56± 0.20**
Group- IV	5.50 ± 0.19ns	4.34± 0.23**	3.33 ± 0.23**
Group- V	5.49 ± 0.29ns	4.17 ± 0.32**	3.47 ± 0.38**
Group- VI	5.35 ± 0.49ns	4.00 ± 0.44**	3.26 ± 0.24**
Group-VII	5.98 ± 0.38ns	2.88 ± 0.32**	3.10 ± 0.42**

The results were expressed as Mean ± SD (n = 6),
ns p>0.05, ** p<0.01, *p<0.05 when compared to STZ treated Negative Controlgroup rats.

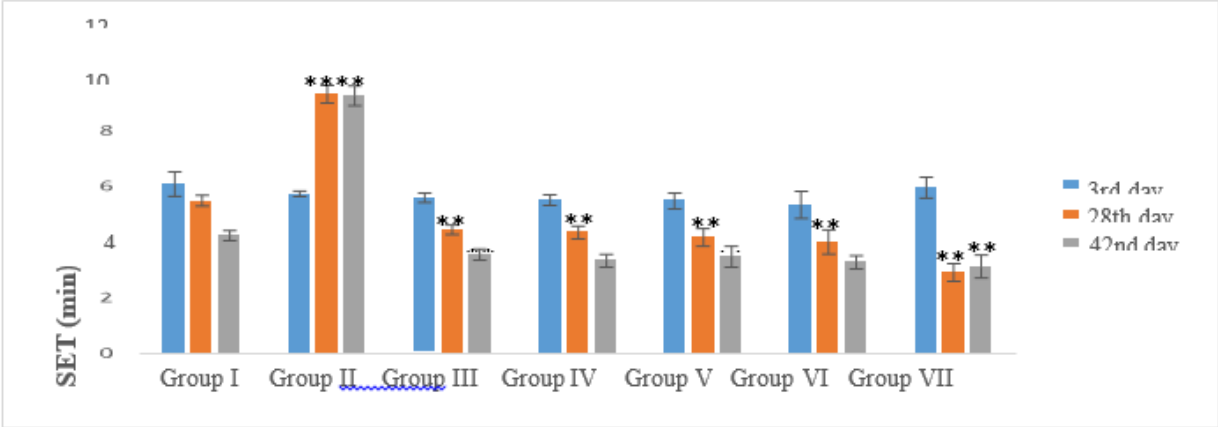

Figure : Swimming Endurance test (SET) on 3rd,28th and 42nd day.

Table 6 and Figure 7 State the effects of STZ on swimming activity of the ratson 3rd , 28th & 42nd day of STZ induction. There was significant decrease ($p < 0.01$) in the immobility of rats in negative control group compared to normal control group of rats on 3rd,28th & 42nd day. This decrease in the immobility confirms the loss of muscle strength in the rats. Group III (High dose of MECI),Group IV (Low dose of MECI), Group V (High dose of WCI), GroupVI (Low dose of WCI) showed significant increase in ($p < 0.01$) in the force swim test compared to Negative control Group.

V. DISCUSSION

Diabetic neuropathy, a well-known, long-term complication of diabetes, which affect almost half of the diabetic population and shows higher morbidity and mortality rate (Amir Aslam et al., 2014). Diabetic neuropathy includes a variety of clinical or subclinical presentations. The reported prevalence of Peripheral Diabetic Neuropathy (PDN) varied from 11% in Rochester, Minnesota, USA to 53.7% in the Middle East .(Amir Aslam et al 2014).One UK study published in 2011 reported that the prevalence of PDN was 21.5% in type 2 diabetes patients and 13.4% in type 1 diabetes patients, resulting in an overall prevalence of 21% . In the large, prospective eurodiab study in 16 European countries, almost one-quarter of type 1 patients developed new onset painful diabetic neuropathy over a seven-year period(Amir Aslam et al., 2014).

Streptozotocin (STZ) and Alloxan (ALX) are the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). STZ has longer half-life (15 min against 1.5 min of alloxan) (A.A. Rossini et al.,1977). This makes it more stable in solution before and after injection into animals. STZ-induced hyperglycemia is relatively more stable and for a longer duration (as much as three months compared to

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alloxan-induced hyperglycemia that can only be sustained for less than a month). Moreover, the mechanism of STZ diabetogenicity is less associated with cellular toxicity, hence, lesser animal mortality. Alloxan on the contrary, induces diabetes by a mechanism characterized by incidences of ketosis, ROS toxicity, and high mortality rate which is particularly a major setback in experimental diabetes studies(T. Szkudelski,2001)

One reason for this is that STZ is more selective to islet beta cells than alloxan which causes severe damage to other cell types which express Glucose Transporter 2 (GLUT2) (systemic toxicity).(S. Lenzen,2008) In addition, compared to alloxan, STZ diabetogenicity is not severely interfered with by blood glucose level. Overall, STZ diabetogenicity is more effective and with lesser variation with animal species (S. Lenzen & R. Munday,1991). So STZ induced diabetic model is preferred for the induction of diabetes in rat which is given intraperitoneally having dose 60 mg/kg.

Prolong diabetes leads to diabetic neuropathy. For confirmation of diabetic neuropathy Tail flick model, Rota rod model and Swimming endurance test models are generally used.(D'Amour et al., 1941).Hence we used this models that confirms DN in rats.

Neuropathic pain in DN is the other most important factor of concern. Literature reveals that estimation of the prevalence of DN varies substantially depending on diagnostic criteria and the intensity of investigation; most studies suggest that about 30-50% of all diabetic people are affected (Anuradha Singh et al.,2013). Pain associated with diabetic neuropathy occurs spontaneously resulting into hyperalgesia or allodynia and are of different in character to that common type due to an injury, burn, pressure, etc (Yadu Nandan Dey & Shankhajit De,2010)

Tail flick model, Rota rod model & Swimming endurance test model are the models used for diabetic neuropathic pain. Among which tail flick model is used to measure the effectiveness of analgesics, by observing reaction to heat.(D'Amour et al., 1941) Rota rod model is used to measure balance, grip strength and motor co-ordination.(Mouzon et al.,2012) Swimming endurance test model is used to measure development of stress (Dominique Sigaudo Roussel et al.,2007).

Herbal medicine is one of the ancient therapies used by humanity(Li JW-H & Vederas JC,2000) During the recent years, people are eager to use herbal medicines due to their lower complications and fewer side effects than synthetic drugs.(Boyd A et al., 2013) The more common plants which are used for the treatment of neuropathic pain are included as: Acorus calamus, Artemisia dracunculus, Butea monosperma, Citrullus colocynthis, Curcuma longa, Crocus sativus, Elaeagnus angustifolia, Ginkgo biloba, Mitragyna speciosa, Momordica charantia (Fatemeh Forouzanfar & Hossein Hosseinzadeh,2018)

Claoxylon indicum contain amino acid, alkaloids, anthraquinone, phenol, saponin,terpenoids,Flavonoides.Hence this plant was selected for screening of Diabetic Neuropathic pain. (D. K Ved et al., 2016). Claoxylon indicum showed the improvement in pain response, body weight and in muscle co-ordination. It also minimised the increased blood glucose level.

The literature reported that in Diabetic Neuropathy, muscle coordination gets disturbed and even that affects swimming Endurance. It is due to slow nerve conduction that leads to abnormalities in biochemical parameters in nervous tissue. This is due to decreased axonal transport and impaired axon regeneration in the nervous system signal transduction cascade (Vinoth prabhu et al.,2011)

Previous investigations suggest that the signal transport is maintained by anticonvulsants and antidepressants. That is why they are used in its management and maintains of the muscle co ordination as well as Swimming Endurance.

Methanolic and aqueous extract at high dose and low dose showed significant ($p< 0.01$) improvement in the muscle co-ordination and swimming Endurance as compared to negative control group.

As the CI having proved anticonvulsants property that may heal in the muscle coordination and Locomotors Behaviour. The drug having the anticonvulsants and antidepressants property shows the improved muscle coordination in the patient suffering from DN.

Investigation suggests that reason behind pain in Neuropathy is due to potential damage to tissues as well as problem associated with the dysfunctioning of one or more nerves which sends pain messages to the brain.

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Reports suggest that analgesics like Tramadol, Morphine is used in the management of pain caused due to DN. They increase the concentration of Nor adrenaline in locus coeruleus and improve the condition against paraesthesiae and allodynia (Yadu Nandan Dey & Ajoy Kumar Ghosh,2010)

Methanolic and aqueous extract at high dose and low dose showed significant ($p< 0.01$) improvement in the analgesic response as compared to negative control group which might be due to repairing of damage to tissues and nerves which sends pain impulses to the brain.

As the CI having proved Analgesic property which improve the condition against pain behaviour which is useful in patients suffering from DN.

Claoxylon indicum showed improvement in muscle co-ordination, swimming endurance and analgesic property.

VI.CONCLUSION

Present study reveals that Claoxylon indicum (CI) is effective in the treatment of DN in rats. The methanolic and aqueous extract of Claoxylon indicum (CI) lowers increased glucose level as well as showed improvement in Body weight in DN rats.

CI showed significant improvement in the muscle coordination activity and also improved analgesic response

Herewith it may be concluded that Claoxylon indicum may be useful in treatment and management of Neuropathic pain induced by DM.

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