IJBPAS, August, 2025, 14(8): 4693-4710

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EXTRACTION AND EVALUATION FOR ANTI DIABETIC NEUROPATHY EFFECTS OF SOME INDIAN PLANT EXTRACTS

METHRI N[1], JAT RK[2] AND BODA R[*3]

1: Research Scholar, SJJT University, Jhunjhunu-churela Road, Jhunjhunu, Rajasthan, India-333001

2: Department of Pharmacology, SJJT University, Jhunjhunu, Rajasthan, India-333001

3: Department of Pharmacology, MLR College of Pharmacy, Dundigal (V, M), Telangana State, India-500088

***Corresponding Author: Dr. Rambabu Boda: E Mail: rambabuboda34@gmail.com**

Received 10th April 2024; Revised 4th May2024; Accepted 7th Sept. 2024; Available online 1st Aug. 2025

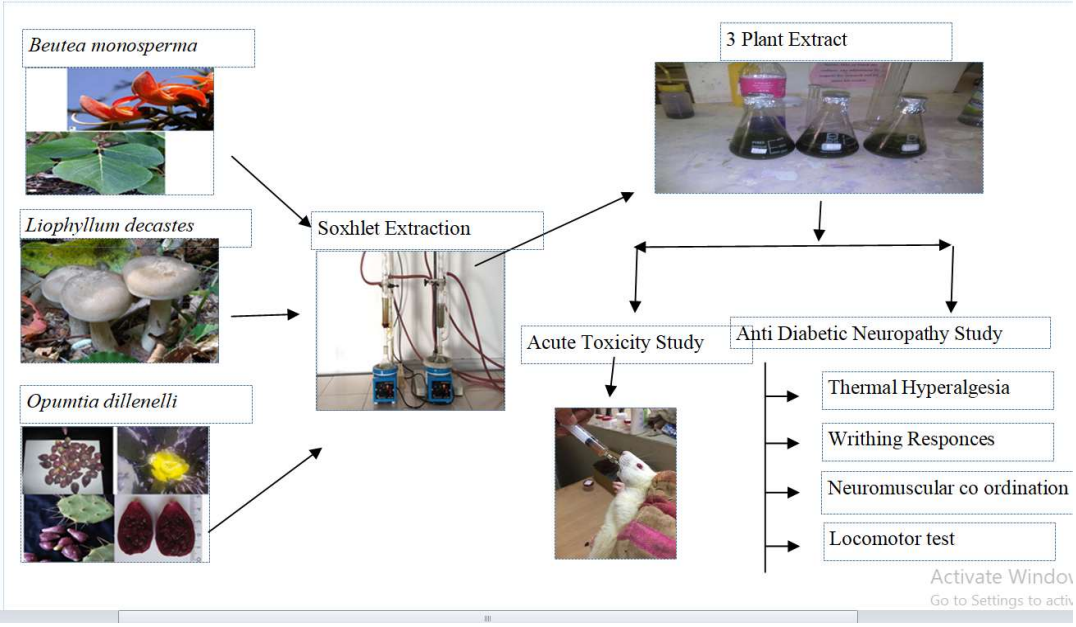
https://doi.org/10.31032/IJBPAS/2025/14.8.9369

ABSTRACT

The importance of Phyto pharmaceuticals in medicine has long been noted, thus it is imperative to look into safe and effective medications for the treatment of metabolic diseases. Diabetes is a metabolic disease associated with changes in the structure and function of several biological systems. The main cause of tissue injury is persistent hyperglycaemia. While peripheral neuropathy and small- and large-vascular disease may also be used to explain diabetes-related organ failure, throughout the past 20 years, the harmful consequences of persistent hyperglycemia have extended beyond angiopathy and neuropathy. A fungus fruit extract of the *Liophyllum dicastes*, a leaf extract of the *Butea monosperma*, and fruit extract of the *Opuntia dillenil* was shown by phytochemical testing. The study findings of *Butea Monosperma* distilled (300 mg/kg) and *Liophyllum decastes, Opuntia dillenelli* on DM rats lured with STZ were correlated with Glibenclamide. diabetes that was created by experimentation and eventually led to diabetic neuropathic problems. Blood glucose levels, body weight, grip strength, writing, mechanical hyperalgesia, spontaneous locomotor (exploratory) test, lipid profile analysis, and thermal hyperalgesia were among the parameters that demonstrated the

drug's protective effects against diabetes and diabetic neuropathy. It was discovered that the liver and sciatic nerve cells of the treated groups showed less inflammation, deterioration, and necrosis. Shown were the antidiabetic properties and diabetic neuropathy of the distilled leaves of *Butea Monosperma*, fruit extract of the *Opuntia dillenil* and a fungus found in *Liophyllum decastes*.

Keywords: Diabetic neuropathy, Liophyllum decastes, Butea Monosperma



INTRODUCTION

Diabetic neuropathy (DN) is a diverse consequence of diabetes mellitus; in type 1 diabetes, it manifests late, while in type 2 diabetes, it may manifest early. The chosen DN animal model should display characteristics seen in human pathophysiology. The pathogenesis of neuropathy is extremely complicated and has been linked to degeneration of both myelinated and unmyelinated sensory fibers, peripheral demyelination, and a reduction in peripheral nerve conduction **[1]**. The DN is dependent on a number of etiological variables, such as autoimmune-mediated nerve damage, microvascular insufficiency, oxidative and nitrosative stress, deficient neurotrophism, and prolonged hyperglycemia **[2, 3]**. It is estimated that diabetic neuropathy and the morbidities it causes cost the US more than $10.9 billion annually **[4]**. The bulk of research on "induced diabetes" in animals reveals that the condition directly causes Neuro degeneration in the hippocampus, which is the first structure to be impacted by Alzheimer's disease-related Neuro

degeneration and is linked to learning and memory **[5]**. The fungus fruit of *Liophyllum decastus*, a leaf of the *Butea monosperma* plant, and fruit extract of the *Opuntia dillenelli* is widely used in medicine for treating a variety of ailments, including bleeding, anticancer, antitumor, analgesic, antibacterial, anthelmintic, and analgesic. Seeds and leaves contain anti-ovulatory and anti-implantation properties and are beneficial as diuretics, astringents, and hemostatics. Numerous animal studies demonstrate the potential of these pharmaceutical drugs, however they were withdrawn from clinical trials either because they were ineffective or because they had negative effects on important organs **[6]**. Thus, there is a great need to create non-pharmacological strategies and alternative medications for the treatment and prevention of diabetes as well as neuropathic alterations linked to Pre diabetes. Understanding the metabolic and molecular pathways of diabetic neuropathy has advanced recently **[7]**. The current focus in diabetic neuropathy with neuro degeneration is on herbal-based treatments.

MATERIALS AND METHODS

Materials:

Plant Collection and Extraction Process:

According to Dr. M. Madhava Chetty of the Department of Biological Sciences at Sri Venkateswara University in Andhra Pradesh, the fungus fruit of *Liophyllum decastes* and the healthy, and fruit extract of the *Opuntia dillenil,* disease-free foliage of *Butea monosperma* have stayed together from the hills of Tirupati, India. Each plant's leaves were chopped into a coarse residue using a chopper after being shade-dried for two weeks. For 24 hours, 500 ml of methanol was continually mixed with 100 g of powdered plant leaves.

Animals:

An Throughout the experiment; named Albino Wistar rats weighing 180–230 g were used. Under normal ecological conditions, they were kept in close proximity to water and pelleted feed. Under the extraction process, 200 millilitres of distilled water were used to extract the leaves of the plant *Butea monosperma*, and fruit extract of the *Opuntia dillenil,* a fungus that grows fruit on *Liophyllum decastes*. Z STZ According to OECD guidelines, the Made Diabetes in Rats experiment was approved, and the I A E Committee and C P C S E A obtained ethical approval.

Acute Toxicity Study:

The acute oral toxicity research was carried out in compliance with OECD guideline - 423 (Acute Toxic Class Method) **[8, 9]**. Acute oral toxicity - describes how albino rats of the same sex (n=3) were utilized and randomly selected for the acute toxicity investigation. Overnight, the animals were given just water to eat and were fasted. After that, the extracts were given orally by

stomach intubation at a dosage of 5 mg/kg body weight, and the patients were monitored for 14 days.

The amount in question is considered hazardous if two of the three animals show signs of death. To validate the hazardous dosage, the same dose is administered again if one animal dies. Higher dosages, such as 50, 300, and 2000 mg/kg body weight, were tried if no mortality was seen. The plastic enclosures held the animals at normal temperature and provided them with free access to water and pelleted nutrition.

METHADOLOGY

Anti-diabetic Neuropathy Activity

Experimental Design

The following protocol was applied to nine groups of six rats each.

Group I: Normal control (saline).

Group II: STZ treated control (150 mg/kg.ip).

Group III: STZ (150 mg/kg.ip) + *Butea Monosperma* leaf extract (150 mg/kg, p.o),

Group IV: STZ (150 mg/kg.ip) + *Iodocea sechellarum* Fruit extract (150 mg/kg, p.o),

Group V: STZ (150 mg/kg.ip) + *Opuntia dillenil* leaf extract (150 mg/kg, p.o).

Group VI: STZ (150 mg/kg.ip) + *Butea Monosperma* leaf extract (300 mg/kg, p.o).

Group VII: STZ (150 mg/kg.ip) + *Iodocea sechellarum* Fruit extract (300 mg/kg, p.o).

Group VIII: STZ (150 mg/kg.ip) + *Opuntia dillenil* Fruit extract (300 mg/kg, p.o).

Group IX: STZ (150 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o).

For six weeks, the medication is delivered orally via catheter each morning. The animals were all given unrestricted access to water but were starved for the whole night after the medication treatment. Blood and urine samples were examined for the following criteria the next morning. As part of the study, the following data were collected: body weight, fasting blood glucose, lipid profile, and serum profile (from trace levels). For each group, the average daily food and drink intake was determined using the swallowed pellets **[10]**.

Development of diabetic neuropathy

A 12-week continuous treatment regimen was used to evaluate the impact of diabetic neuropathy. Using thermal hyperalgesia, the number of twitch responses in ten minutes, mechanical hyperalgesia or grip strength test, acto photometer, and rota rod test, the development of diabetic neuropathy was precisely documented in all groups. Blood glucose levels, body weight, writing profile analysis, and thermal hyperalgesia were among the parameters that demonstrated the drug's protective effects against diabetes and diabetic neuropathy.

Oral glucose tolerance test

Nine groups of six rats apiece were created from the rats. Prior to therapy, every animal was fasted. Group I was maintained as the

vehicle control group, getting 5% between 80 p.o.; Group II was given STZ only; Group III, IV, and V got 150 mg/kg of methanolic extract; Group VI, VII, and VIII received 300 mg/kg of methanol extract; and Group IX was given STZ with the standard medication Glibenclamide (5 mg/kg). Only in the car in each instance Thirty minutes after the medication was administered, rats in Groups III to VIII received a p. o. treatment of glucose (3 g/kg). Before the medication was administered, thirty, ninety, and one hundred fifty minutes after the glucose was administered, blood samples were taken via retro orbital sinus puncture. Using a glucose detection kit, serum glucose levels were determined right away. (Sigma Diagnostic, Mumbai, India).

Collection of blood sample and blood glucose determination

Urinary and blood samples All animals were maintained in metabolic cages for a full day following the conclusion of the medication treatment. While fasting during the night, all animals had unrestricted access to water. The next morning, under mild ether anaesthesia, a retro orbital puncture was used to obtain a blood sample. From the rat's tip, blood samples were obtained once a week until the study's conclusion, or every two weeks. On days 4, 7, 10, and 14 of the trial, measures of body weight and fasting blood glucose levels were taken. Using glucose test strips and an electronic blood glucose meter (One-Touch), blood glucose may be measured. Day 14 saw the collection of blood under light ether anaesthesia from the retro-orbital plexus of rats who had fasted overnight fasting blood glucose was determined, Serum was separated and analysed for Serum was separated and analysed for Estimation of Cholesterol **[11, 12]**, serum Triglycerides **[13]** using the enzymatic DHBS colorimetric method, serum HDL-Cholesterol **[14]**, serum ALKALINE PHOSPHATASE **[15]** SGOT/AST **[16, 17]**, SGPT/ ALT **[18, 19]**, Estimation of Serum Total Proteins **[20, 21]**, Glucose **[22]** were estimated by the hydrolysed phenol-amino-antipyrine method.

Analytical Methods:

Fasting Blood glucose Level

Following fifteen test days, all groups of rats were fasted, and blood samples were obtained from the tail area to measure blood glucose levels. The findings demonstrated that the diabetes control group's fasting blood glucose levels were considerably higher than those of the normal control group over the study period (day 0–15) **[23]**. From day 0 to day 15, there was a statistically significant drop in blood glucose levels in the Glibenclamide (5 mg/kg) group as compared to the diabetic control group. Similarly, as compared to the diabetic control group, the treated rats' blood glucose levels were considerably lowered by

the fruit extract of the *Opuntia dillenil,* methanolic extract of *B. monosperma* and *Iodocea sachellarum* fruits (150 and 300 mg/kg). The Glibenclamide group had the blood glucose level that was statistically lowest. Groups IV, V, and VI showed a dose-dependent impact on fasting blood glucose levels (Table3).

Body weight

Body weight was assessed at the beginning of animal selection, seven days into therapy, when experimental diabetes was induced, and at the conclusion of the study. Water consumption was evaluated using calibrated water bottles, and food intake was determined by counting the quantity of pellets ingested daily during the trial **[10]**.

Thermal Hyperalgesia

The animals in each group were individually placed in an analgesic device with a heating plate (Columbus Instruments, USA) in the Eddy's hot plate technique test, with the temperature set to 55°C. A pain threshold index was computed as soon as the first indications of paw squeezing or leaping appeared. After ten seconds, the experiment was discontinued to prevent damage to the animals' paws. This investigation was assessed at weeks 2, 4, 8, and 12 **[24]**.

Mechanical hyperalgesia

A measuring needle that had been bent 90 degrees was applied to the hind leg of the pinprick test rat; only one puncture was done to make sure the needle did not pierce the skin. In every group, reflex withdrawal responses were noted and observed. With a 20-second cut off, the length of the paw withdrawal was measured in seconds. After two, four, eight, and twelve weeks, the outcomes were documented for each group **[25]**.

Writhing responses

The acetic acid writing method was also utilized to evaluate neuropathic pain in all animal groups. 1% v/v acetic acid produced in distilled water was administered to the animals. The rats received an intra-peritoneal injection of 1% acetic acid at a rate of around 0.1 ml/10 g body weight, 60 minutes after the dosages were administered. After 30 minutes of recording, the episodes were reviewed, and the number of stretching motions—including those involving the torso, the back, and the limbs—was recorded **[26]**.

Neuromuscular coordination test

After 2, 4, 8, and 12 weeks, neuromuscular coordination in all groups of rats was measured using a Rota rod (motor coordination). The rats were positioned at a rotating speed of 25 revolutions per minute on a Rota rod device. Each group's fall-off time was noted throughout the first five minutes of walking **[27]**.

Spontaneous Locomotor test

An act photometer assisted in a spontaneous motor exploration test that was conducted in order to document and comprehend the

animal behaviour (exploratory actophotometer). A closed acto-photometer (30 30 30 cm) including photocells on the exterior wall was utilized to house the animals. A computer counter tracks the beam's disruptions **[28, 29]**.

Biochemical analysis: At the conclusion of the 14-day testing period, the animals were decapitated and slaughtered. After collecting the blood, the serum was separated using centrifugation for ten minutes at 3000 rpm. In rats that were drug-treated, diabetes-induced, and normal, the following characteristics of diabetic complications—cardiomyopathy, neuropathy, and nephropathy—were examined. Parameters of diabetic nephropathy estimated biochemically.

RESULTS

Phytochemical Screening

Several qualitative phytochemical screening assays revealed the presence of carbohydrates, glycosides, fixed oils, tannins, phyto sterols, flavonoids, and alkaloids in the methanolic extract of *Butea monosperma* leaves. The methanolic extract under investigation has a greater concentration of flavonoids and glycosides.

Several qualitative phytochemical screening assays revealed the presence of carbohydrates, glycosides, flavonoids, fixed oils, lipids, tannins, phytosterols, and alkaloids in the methanolic extract of *Liophyllum decastes* mushroom fruit. These fruits have increased flavonoid and glycoside contents.

The methanolic extract of *Opuntia Dillenelli* fruit included the following: carbohydrates, glycosides, flavonoids, fixed oils, lipids, tannins, phytosterols, and alkaloids, according to many qualitative phytochemical screening tests. Fruits with higher flavonoid and glycoside concentrations are these ones.

Fasting blood glucose

The recorded outcomes are displayed in **Table 1**. On the 28th day of the trial, the blood glucose levels of the treated groups were found to have decreased; in groups III and VI, they were 123.6±3.81 and 190.3±19.65 mg/dl, respectively. When compared to group II's (the diabetic control group) findings from week 12, the results are noteworthy.

Body weight

From Day 0 to Week 28, the rats in Groups III and IV put on weight. The weight increase contrasts with the weight loss symptom associated with diabetes, indicating an improvement in the treated groups' health state. The diabetic group's average weight was 126.9 ± 0.33 g, whereas the other groups' average weights were 201.1 ± 0.27 and 211.7 ± 0.20 g. **Table 2** presents the findings.

Thermal Hyperalgesia: Eddy's hot plate method

Table 3 presents the findings. At Days 14 and 21, respectively, treatment group IV's mean reaction latency was 4.28 s and 4.54 s in groups IV and V. When compared to the Day 28 results of group II (the diabetes control group), the results are noteworthy.

Mechanical hyperalgesia: pinprick test

Table 4 compares the outcomes. When compared to Day 28, Group VI's and Group VII's replies improved to 4.16 and 3.71, respectively. Data include the answers of the diabetes control group (Group II) from Day 0 to Day 28.

Writhing responses

In ten minutes, Group VII displayed an average of 13.34 replies, and Group IV displayed an average of 14.56. The results were very significant when compared to Day 28 answers of the diabetes control group (Group II). **Table 5** presents the findings.

Neuromuscular coordination test (motor coordination): rota rod test

Table 6 presents the findings. The animals in groups IV and VII showed improvements in neuromuscular coordination of 76.57 and 78.87, respectively. The results of treated groups IV and VII, expressed as mean average (SEM), when analysed by one-way ANOVA followed by Tukey-Kramer multiple comparison test, showed $P < 0.001$ significance compared with the diabetic control groups (group II). At Day 28, the results were compared with the results of the diabetic control group (group II). This highlights the healing and shielding properties of chlorogenic acid.

Spontaneous Loco motor (exploratory) test:

Table 7 displays the findings of the Actophotometer. After taking dosages for four weeks for 28 days, the exercise activity of the group improved to 89.13 and 87.43 in Group IV and Group VII, respectively. As the outcomes were contrasted with the replies of Group II, the diabetes control group in the 7.

Table 1: Fasting Blood glucose Level

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% Nacl	91.6± 4.2	92.0± 1.7	93.3± 2.5	92.7± 4.4	90.4±2.3
Group II	STZ	150mg/kg	291.0± 5.13	287.0±9.77	283.3±6.56	281.8±7.26	278.5±12.3
Group III	STZ+ BME	150+150	290.3± 6.73	204.5± 13.03	187.0±15.47	146.5± 14.57	123.6±3.81
Group IV	STZ+ISE	150+150	282.7± 9.18	260.6±5.46	245.1± 3.71	242.3± 3.37	211.0±7.61
Group V	STZ+ODE	150+150	281.83± 9.6	251.0±7.72	221.8±19.65	175.3± 18.35	147.3±6.28
Group VI	STZ+ BME	150+300	271.67± 9.18	251.8±9.17	234.17±3.71	222.8± 3.37	190.3±19.65
Group VII	STZ+ISE	150+300	274.7± 7.18	258.6±5.23	239.1± 4.21	234.3± 3.17	210.0±6.51
Group VIII	STZ+ODE	150+300	289.84± 8.6	249.0±6.52	220.8±14.35	171.3± 13.37	138.3±5.18
Group IX	Glibenclamide	5 mg/kg	293.83± 5.02	260.83±9.17	164.67±9.52	126.8± 6.85	103.8±3.76

Values are expressed as Mean SEM; n¼6. One-way ANOVA; followed by Tukey-Kramer multiple comparisons test: a P< 0.001incomparisonwithnormalcontroland ***P < 0.001 in comparison with the diabetic control

Table 2: Comparision of Body weight

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% NaCl	121.2 ± 0.28	122.8 ± 0.20	123.6 ± 0.25	125.4 ± 0.47	127.5 ± 0.45
Group II	STZ	150mg/kg	129.8 ± 0.49	127.6 ± 0.38	126.4 ± 0.49	127.2 ± 0.38	126.9 ± 0.33
Group III	STZ+ BME	150+150	128.3 ± 0.33	131.6 ± 0.93	133.4 ± 0.36	134.1 ± 0.41	201.1 ± 0.27
Group IV	STZ+ISE	150+150	128.5 ± 0.28	128.3 ± 0.36	129.2 ± 0.16	129.4 ± 0.22	211.7 ± 0.20
Group V	STZ+ODE	150+150	127.8 ± 0.27	128.5 ± 0.28	128.2 ± 0.24	127.9± 0.27	126.7 ± 0.3
Group VI	STZ+ BME	150+300	128.5 ± 0.29	127.8 ± 0.31	126.4 ± 0.23	125.7 ± 0.22	125.9 ± 0.27
Group VII	STZ+ISE	150+300	129.5 ± 0.26	129.1 ± 0.32	128.4 ± 0.17	128.1 ± 0.12	128.7 ± 0.15
Group VIII	STZ+ODE	150+300	128.6 ±0.24	128.2 ± 0.120	127.2 ± 0.21	127.3± 0.19	126.4 ± 0.4
Group IX	Glibenclamide	5 mg/kg	134.3 ± 0.31	132.4 ± 0.38	133.7± 0.43	134.4 ± 0.31	135.3 ± 0.22

Values are expressed as Mean SEM; n ¼ 6. One-way ANOVA; Tukey-Kramer multiple comparisons test: aP< 0.001 in comparison with normal control; and ***P < 0.001 in comparison with the diabetic control

Table 3: Thermal Hyperalgesia

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% NaCl	4.1±1.8	4.18 ±0.3	4.33± 1.2	4.53 ±2.1	4.94 ±2.8
Group II	STZ	150mg/kg	6.65 ± 2.3	7.16 ± 0.2	8.24± 0.12	9.20±0.9	9.62±0.1
Group III	STZ+ BME	150+150	7.42± 0.2	6.81± 2.1	5.11± 0.21	4.81 ±1.3	4.10 ±1.1
Group IV	STZ+ISE	150+150	6.01± 0.1	5.01± 0.2	4.22 ±2.9	4.54± 0.8	4.23± 0.8
Group V	STZ+ODE	150+150	6.05± 0.4	4.92 ±1.3	4.98± 0.6	4.31 ±2.93	4.10 ±2.8
Group VI	STZ+ BME	150+300	6.06± 0.8	5.09±0.13	4.95 ±1.3	4.10 ± 0.6	3.78± 0.1
Group VII	STZ+ISE	150+300	6.08± 0.1	6.01± 0.1	5.12 ±2.3	3.42± 0.8	3.12± 0.6
Group VIII	STZ+ODE	150+300	6.09± 0.4	5.02 ±1.4	3.68± 0.5	3.81 ±2.71	4.01 ±2.8
Group IX	Glibenclamide	5 mg/kg	6.26± 0.22	5.32±0.21	5.01 ±0.12	3.32± 0.12	2.22± 0.12

Values are expressed as Mean SEM; n¼ 6. One-way ANOVA; followed by Tukey-Kramer multiple comparisons test: a P< 0.001 in comparison with normal control; ***P < 0.001 in comparison with the diabetic control

Table 4: Mechanical Hyperalgesia

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% Nacl	2.23± 0.82	2.31± 0.16	1.19± 0.66	2.21± 0.31	1.14± 0.31
Group II	STZ	150mg/kg	5.16 ±1.36	6.86 ±1.04	6.71± 0.48	7.78±1.04	6.78±1.04
Group III	STZ+ BME	150+150	6.23± 0.64	7.89±0.21	8.61 ±1.39	4.36 ±1.32	4.16 ±1.32
Group IV	STZ+ISE	150+150	6.64 ±1.06	7.84± 1.23	4.25±0.12	3.41± 0.62	2.91± 0.62
Group V	STZ+ODE	150+150	5.0 ± 0.64	6.79±0.21	7.41 ±1.39	5.16 ±1.32	5.24 ±1.32
Group VI	STZ+ BME	150+300	6.84 ±1.06	7.8 ± 1.23	5.05±0.12	4.6 ± 0.62	4.1 ± 0.62
Group VII	STZ+ISE	150+300	6.24 ±1.02	7.14± 1.13	5.35±0.22	4.41± 0.63	3.71± 0.62
Group VIII	STZ+ODE	150+300	5.04± 0.61	6.86± 0.24	7.57 ±1.29	5.16 ±1.22	4.84 ±1.32
Group IX	Glibenclamide	5 mg/kg	5.8 ± 1.19	6.9 ± 1.02	7.92±0.28	5.99 ±0.37	6.99 ±0.37

Values are expressed as Mean SEM; n ¼ 6. One-way ANOVA; Tukey-Kramer multiple comparisons test: aP< 0.001 in comparison with normal control; and ***P < 0.001 in comparison with the diabetic control.

Table 5: Writhing Responces

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% NaCl	16.4±2.2	13.2±2.02	12.13±1.7	9.6±1.2	9.4±1.1
Group II	STZ	150mg/kg	25.1 ±0.2	26.8±1.5	26.9 ±0.5	28.2±0.4	29.3±0.3
Group III	STZ+ BME	150+150	21.1±0.3	22.64±1.3	21.4 ±2.8	17.53±0.7	15.4±0.81
Group IV	STZ+ISE	150+150	21.27±0.9	19.29±2.1	19.18±2.8	15.9±1.30	14.56±1.4
Group V	STZ+ODE	150+150	18.27±2.7	21.29±2.1	19.18 2.9	17.92±1.1	16.7±1.41
Group VI	STZ+ BME	150+300	21.20±0.3	32.54±1.3	21.41±2.2	19.52±0.7	17.82±0.7
Group VII	STZ+ISE	150+300	22.17±0.9	19.19±1.1	18.14±1.8	14.6±1.20	13.34±1.0
Group VIII	STZ+ODE	150+300	18.17±1.7	20.19±2.0	18.19±2.4	16.82±1.0	14.9±1.21
Group IX	Glibenclamide	5 mg/kg	24.1±1.68	25.13±1.1	24.34±0.9	19.94±0.4	18.7±0.45

Values are expressed as Mean SEM; n ¼ 6. One-way ANOVA; Tukey-Kramer multiple comparisons test: aP< 0.001 in comparison with normal control; and ***P < 0.001 in comparison with the diabetic control.

Table 6: Neuromuscular coordination

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% NaCl	121.41 ±1.1	124.2 ±1.1	127.21±1.0	128.13±6.0	129.93±6.0
Group II	STZ	150mg/kg	34.31 ±2.1	25.15 ±5.61	19.34±5.61	11.12±6.13	13.12±6.13
Group III	STZ+ BME	150+150	64.12 ±2.11	71.12±6.12	79.02±8.31	104.23±4.2	98.13±4.2
Group IV	STZ+ISE	150+150	46.50±6.11	56.31 ±6.92	68.23±6.32	75.27±2.13	76.57±2.13
Group V	STZ+ODE	150+150	59.12 ±2.11	69.12±6.12	88.02±8.31	109.23±4.2	119.03±4.2
Group VI	STZ+ BME	150+300	59.50±6.11	71.31 ±6.92	79.23±6.32	83.27 2.13	92.27±2.13
Group VII	STZ+ISE	150+300	45.40±6.11	57.21 ±5.92	69.32±6.32	77.24±2.13	78.87±2.13
Group VIII	STZ+ODE	150+300	58.13 ±2.11	70.12±6.12	89.12±8.31	106.13±4.2	120.03±4.2
Group IX	Glibenclamide	5 mg/kg	62.31±6.14	89.13 ±1.13	104.62±5.3	109.14±1.3	110.24±1.3

Values are expressed as Mean SEM;n¼6.One-wayANOVA;Tukey-Kramer multiple comparisons test:P<0.001in comparison with normal control;*P<0.001in comparison with the diabetic control.**

Table 7: Spontaneus locomotor test

S. No.	Treatments	Dose	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	N Saline	0.9% Nacl	124.5±8.7	123.8±7.3	122.9±8.5	121.6±9.5	120.4±9.5
Group II	STZ	150mg/kg	28.3±7.2	23.98±5.3	19.17±6.8	13.33±6.7	14.53±6.7
Group III	STZ+ BME	150+150	49.67±6.2	74.24±8.3	92.63±5.5	108.3±3.6	115.3±3.8
Group IV	STZ+ISE	150+150	52.67±6.0	64.2 ± 6.2	79.17±7.2	92.13±5.2	89.13±5.2
Group V	STZ+ODE	150+150	46.67±6.2	68.24±8.7	89.63±5.7	112.3±3.1	110.2±3.3
Group VI	STZ+ BME	150+300	53.67±6.2	65.2 ± 6.5	82.17±7.2	95.13±5.8	94.83±5.8
Group VII	STZ+ISE	150+300	51.43±6.0	65.2 ± 6.2	78.14±7.2	91.64±5.2	87.43±5.2
Group VIII	STZ+ODE	150+300	45.66±6.2	69.84±8.1	86.24±5.7	111.3±3.1	108.2±3.3
Group IX	Glibenclamide	5 mg/kg	39.17±6.4	61.67±6.2	70.26±5.2	94.17±5.1	89.20±5.1

Values are expressed as MeanSEM; n¼ 6. One-way ANOVA; followed by Tukey-Kramer multiple comparisons test: aP< 0.001 in comparison with normal control; ***P < 0.001 in comparison with the diabetic control

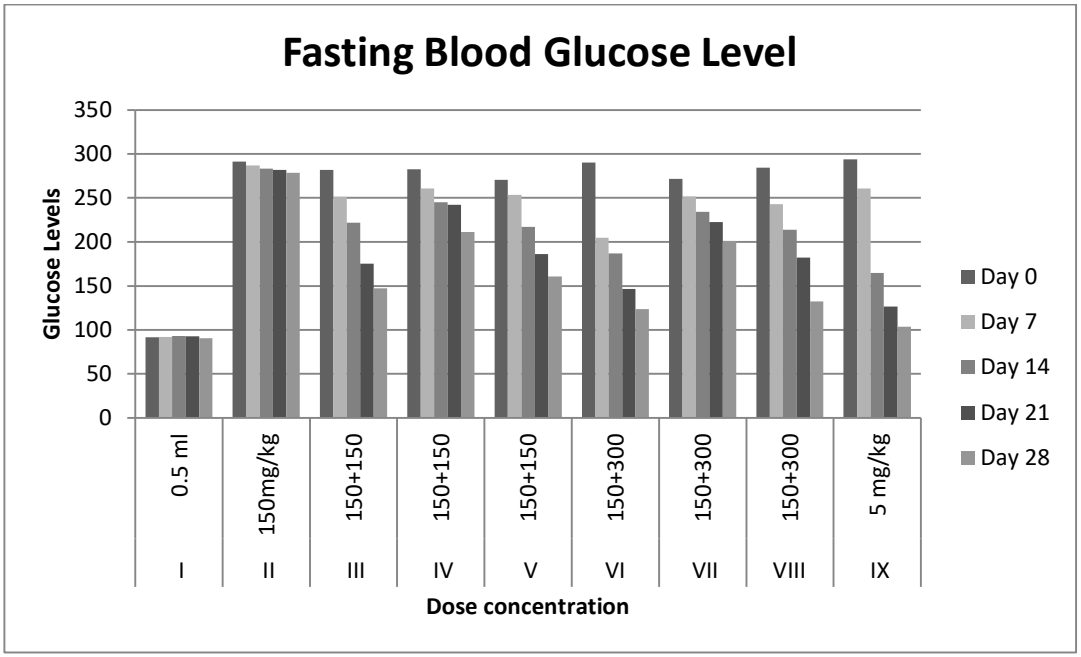


Figure 1: Results showed that Fasting Blood Glucose levels were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

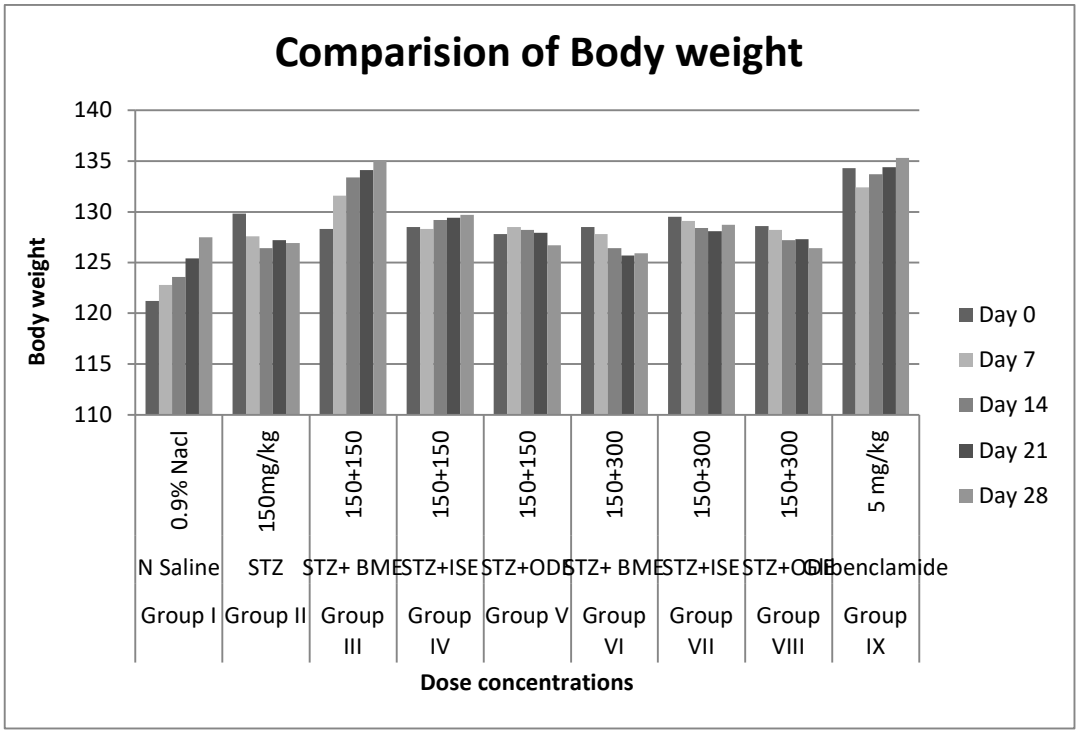


Figure 2: Results showed that Comparision body weight were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

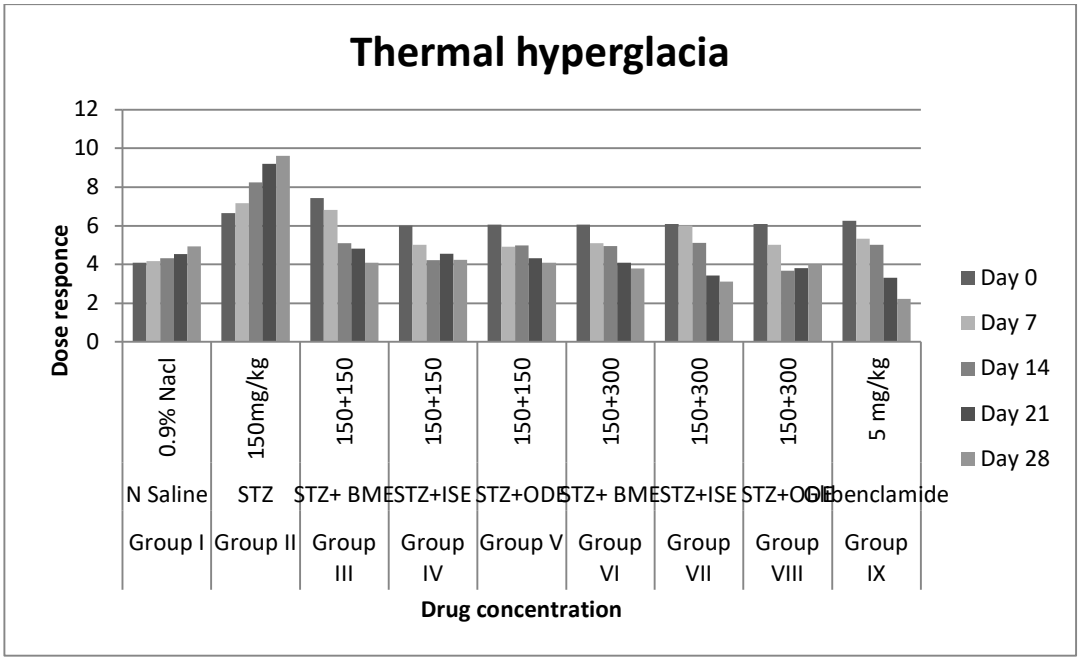


Figure 3: Results showed that Thermal Hyperalgesia were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

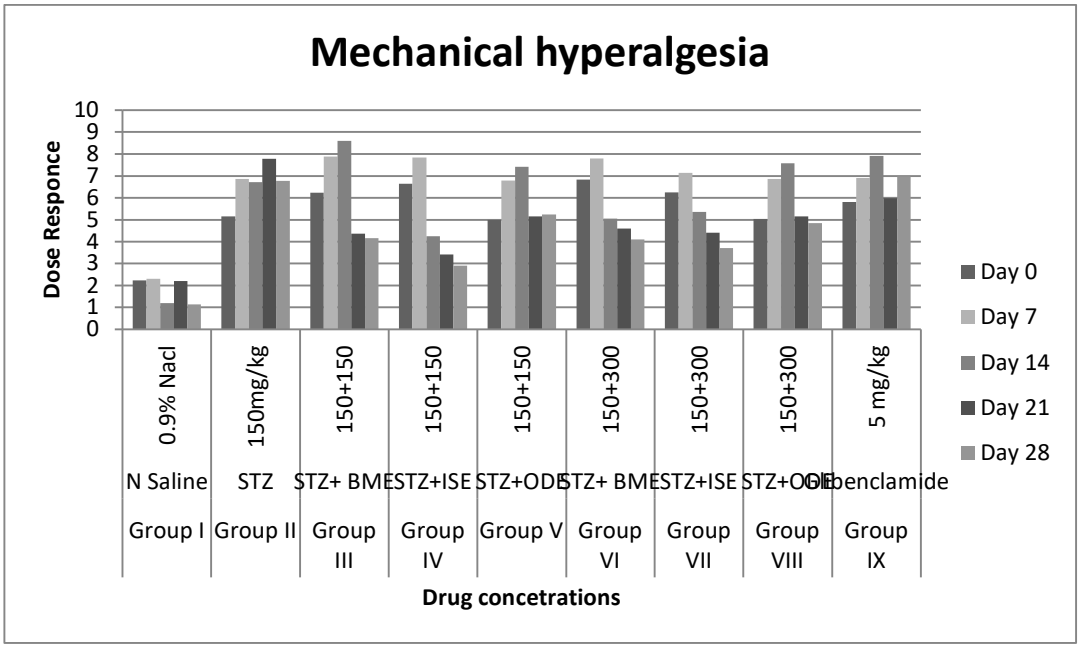


Figure 4: Results showed that Mechanical hyperalgesia were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

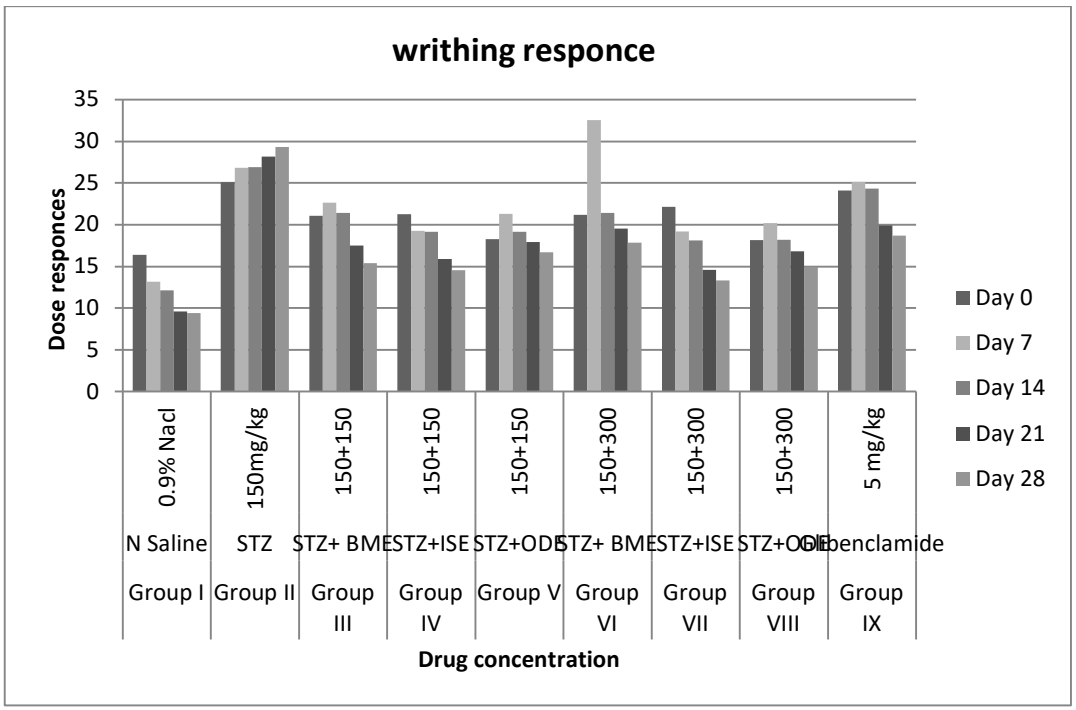


Figure 5: Results showed that writhing responces levels were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

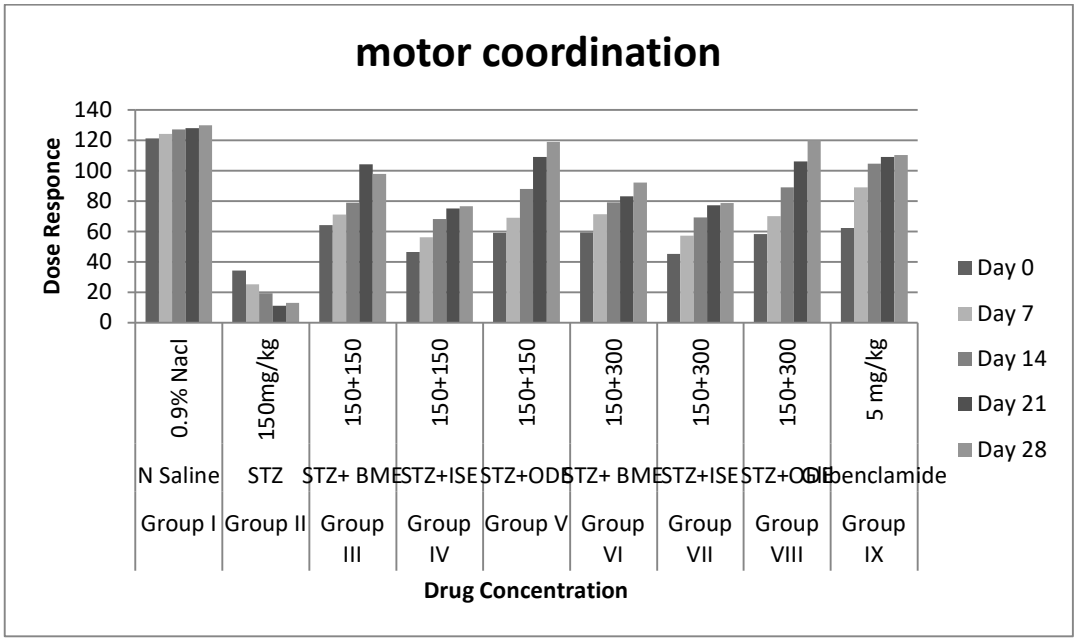


Figure 6: Results showed that motor cordination levels were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

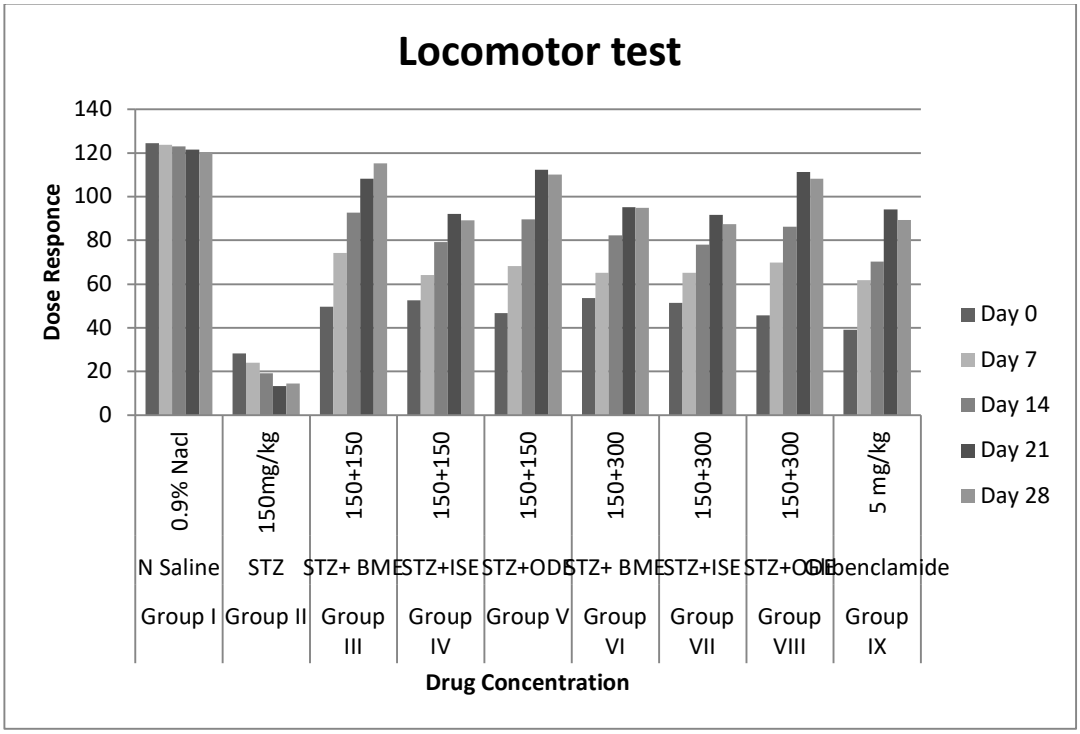


Figure 7: Results showed that locomotor testlevels were significantly elevated in the diabetic control rats. The values are expressed in terms of mean ± SEM (n = 9)

DISCUSSION

The findings of this study demonstrate that in Wistar rats, experimentally induced diabetes and diabetic neuropathy can be reduced by chlorogenic acid. Thus, this relief is explained by the fact that experimentally generated hyperglycaemia and diabetic neuropathy are attenuated by chlorogenic acid that is extracted from the fruit extract of Opuntia dillenelli, leaves of *Butea monosperma*, the fungus fruit of the *Liophyllum decastes*. About 50% of people with diabetes have been reported to have diabetic neuropathy, a widely recognized consequence of the disease. This issue seems to be mostly brought on by the oxidative stress brought on by free radicals that is seen with the onset of diabetic neuropathy.

When compared to the control rats in this investigation, the diabetic rats displayed much longer tail and paw retraction latencies. This implies that the Wistar rats had mechanical and thermal hyperalgesia, which slowed reaction times and got worse over the course of the research. After 12 weeks of treatment, responses to thermal algesia, twitching, and pinpricks improved in all groups, with group V showing the greatest improvement, underscoring the neuro protective impact of chlorogenic acid. In comparison to the diabetes control groups, the therapy groups also shown improvements in grip strength, rotarod responses, and movement sequences captured by the actophotometer. Animals with diabetes eventually exhibit sluggish behaviours, nerve irritation that results in limb discomfort, and delayed movement reactions. As the diabetes worsens diabetic neuropathy develops, the animals lose activity **[30]**.

According to this research, there has been a noticeable decline in diabetic neuropathy. Over the course of four weeks, there was a noticeable drop in glucose levels in this research when compared to the control group. This finding demonstrates the anti-diabetic properties of the fruit of the *Opuntia dillenil,* leaves of *Butea monosperma,* a kind of *Liophyllum dicastes* fungus. According to experimental findings, giving Wistar rats the leaves of *Butea monosperma*, the fruit of the fungus *Liophyllum dicastus*, and fruit of the *Opuntia dillenelli*, had a mitigating impact on the diabetes and diabetic neuropathy caused by streptozotocin. We tracked the animals' body weight to verify the isolated action of chlorogenic acid since weight loss is a defining criterion in the diagnosis of diabetes. Diabetes is characterized by decreasing body weight, which was observed in the animals in the control group; in contrast, the treated

group's body weight increased. This demonstrates how the treated groups' healing effects **[31]**.

Additionally, this study demonstrated a substantial association between the effects of diabetic neuropathy in rats with diabetes and the notable harm that was shown in the rats over a 12-week period. An significant contributing element to the development of diabetic neuropathy and hyperglycaemia is elevated inflammatory levels **[32]**. In chronic diabetes, there is an overexpression of inflammatory biomarkers, which is the reason for cell malfunction and death.

CONCLUSION:

This study validated the traditional assertion of the tribal people as well as the Neuro protective activity and Antidiabetic impact of *Butea monosperma* leaves and *Liophyllum decastes* mushrooms and fruit of the *Opuntia dillenil* in Wistar rats through antioxidant pathways.

Acknowledgement:

The author is Grateful to SJJT University, Churela, Rajasthan.

Conflict of Interest

The authors clearly point out that there is no conflict.

REFERENCES:

[1] Dyck PJ, Zimmerman BR, Vilen TH. 1988. Nerve glucose, sorbitol, myoinositol and fiber degeneration and regeneration in diabetic neuropathy. N Engl J Med. 1;319(9):542-8.Doi: 10.1056/NEJM198809013190904.

[2] Vinik A, Mehrabyan A. 2004. Diabetic neuropathies. A complete review of the diagnosis and treatment of diabetic neuropathy, with useful tables and figures that aid in the differential diagnosis and correct selection of treatment. Med Clin North Am. 88(4):947-99. doi: 10.1016/j.mcna.2004.04.009.

[3] Boulton AJ, Vinik A, Arezzo J. 2005. for the American Diabetes Association. Position statement: diabetic neuropathies. Diabetes Care. 28(4):956-62. doi: 10.2337/diacare.28.4.956.

[4] Gordois A, Scuffham P, Shearer A *et al*., 2003. The health care costs of diabetic peripheral neuropathy in the US. Diabetes Care. 26(6):1790-5. doi: 10.2337/diacare.26.6.1790.

[5] Korf ES, White LR, Scheltens P. *et al*., 2006. Brain aging in very old men with type 2 diabetes: the Honolulu-Asia aging study. Diabetes Care. 29(10):2268-74. doi: 10.2337/dc06-0243.

[6] Veves A, Backonj M, Malik RA. 2008. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. Pain Med. 9(6):660-74. doi:

10.1111/j.1526-4637.2007.00347.x.

[7] Obrosova IG. 2009. Diabetic Painful and Insensate Neuropathy: pathogenesis and Potentialtreatments.Neurotherapeutics.6(4):638-47.doi: 0.1016/j.nurt.2009.07.004.

[8] OECD. Test No. 420: Acute Oral Toxicity – Fixed Dose Procedure. Paris: (2002). https://Doi.org/10.1787/9789264071049-en.

[9] Oecd, 2001. OECD Guideline 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD guideline for the testing of chemicals. France, Paris, pp. 1–14. https://doi.org/10.1787/20745788.

[10] Williams, F., Saffran, M. 2020. Insulin in the drinking water of rats. Heliyon. 6(10): e05137. doi: 10.1016/j.heliyon.2020.e05137.

[11] Slater T F, Sawyer BC. 1971. The stimulatory effects of carbon tetrachloride and other halogen alkanes on per oxidative reactions in rat liver fractions in vitro. General features of the systems used. Biochem J. 123(5):805-14. Doi: 10.1042/bj1230805.

[12] Abourbih S, Falcon KB, Joseph L. 2009. Effect of fibrates on lipid profiles and cardio vascular outcomes. a systematic review. Am J Med. 122(10):962-968. Doi: 10.1016/j.amjmed.2009.03.030.

[13] Davidson MH. 2000. Safety profiles for the HMG-CoA reductase inhibitors: treatment and trust. Drugs. 61(2):197-206. Drugs. 61(2):197-206. Doi: 10.2165/00003495-200161020-00005.

[14] Joy TR, Hegele RA. 2009. Narrative review: Statin-related myopathy. Ann Intern Med. 16;150(12):858-68. Doi: 10.7326/0003-4819-150-12-200906160-00009.

[15] Brugts JJ, Yetgin T, Hooks SE, *et al*, 2009. The benefits of statins in people without established cardio vascular disease but with cardio vascular risk factors: meta-analysis of randomised controlled trials. BMJ. 30:338:b2376. Doi: 10.1136/bmj.b2376.

[16] Murphy SA, Cannon CP, Wiviott SD, *et al*, 2007. Effect of intensive lipid lowering therapy on mortality after acute coronary syndrome: a patient level analysis of the Aggrastat to Zocor and Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infraction 22 trials. Am

J Cardiol.1;100(7):1047-1051. Doi: 10.1016/j.amjcard.2007.04.053.

[17] Hardman, Lombard, Gilman. 2023. Goodman & Gilman's pharmacological basis of therapeutics. Mc Graw Hill medical publishers. 10: 977-986. https://accessmedicine.mhmedical.com/content.aspx.bookid=3191&sectionid=265824603.

[18] Lukacinova A, Mojzis J, Benacka R, *et al*, 2008. Preventive effects of flavonoids on alloxan induced diabetes mellitus in rats. Acta Vet. Brno. 77: 175-182 https://doi.org/10.2754/avb200877020175.

[19] Claudia ENM, Julius EO, Dogbert T, *et al*, 1996. Anti diabetic and Hyperlipidemic effects of Laportea ovalifolia (Urticaceae) in alloxan-induced diabetic rats. Afric J of Trad Complement and Altern Medic. 3: 1, 36-43.

[20] Leonard Tedong, Théophile Dimo, Paul Desire Djomeni Dzeufiet *et al* 2006. Anti hyperglycemic and renal protective activities of Anacardium occidentale (anacardiaceae) leaves in streptozotocin induced diabetic rats. Afric J of Trad Complement and Altern Medic. 3(1): 23-35. DOI:10.4314/ajtcam.v3i1.31136.

[21] Faizal P, Suresh S, Satheesh Kumar R, *et al*, 2009. A study on the hypoglycaemic and Hyperlipidemic effects of a ayurvedic drug RAJANYAMALAKADI in Diabetic patients. Ind J of Clinic Biochem. 24:1, 82-87. https://Doi.org/10.1007/s12291-009-0014-1.

[22] Froude T.S, Y.S. Medeiros. 2008. Animal models to test drugs with potential anti diabetic activity. J of Ethnopharmacology. 115: 173-183. http://dx.Doi.org/10.1016/j.jep.2007.10.038.

[23] Cotter, M.A., Cameron, N.E., 2003. Effect of the NAD(P)H oxidase inhibitor, apocynin, on peripheral nerve perfusion and function in diabetic rats. Life Sci. 22;73(14):1813-24. doi: 10.1016/s0024-3205(03)00508-3.

[24] Kuhad, A., Chopra, K., 2009. Tocotrienol attenuates oxidative-nitrosative stress and inflammatory cascade in experimental model of diabetic neuropathy. Neuropharmacology. 57(4):456-62.doi: 10.1016/j.neuropharm.2009.06.013.

[25] Kanaan, S.A., Saade, N.E., Haddad, J.J. *et al*., 1996. Endotoxin-induced local inflammation and

hyperalgesia in rats and mice: a new model for inflammatory pain. Pain. 66(2-3):373-9. doi: 10.1016/0304-3959(96)03068-0.

[26] Zhang, QW., Lin, LG. & Ye, WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 13, 20 (2018). https://doi.org/10.1186/s13020-018-0177-x.

[27] Carter, R.J., Lione, L.A., Humby, T. *et al*., 1999. Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci.15;19(8):3248-57.doi: 10.1523/JNEUROSCI.19-08-03248.1999.

[28] Anjaneyulu, M., Chopra, K., 2004. Quercetin attenuates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats. Indian J. Exp. Biol. 42, 766–769. PMID: 15573524.

[29] JunXiea, WeiSongb, Xiaochun Lianga, *et al*., 2020. Protective effect of quercetin on streptozocin-induced diabetic peripheral neuropathy rats through modulating gut microbiota and reactive oxygen species level, Biomed Pharmacother. 127:110147. doi: 10.1016/j.biopha.2020.110147.

[30] Salehi B, Ata A, V. Anil Kumar, *et al*. 2019. Antidiabetic potential of medicinal plants and their active components. Biomolecules. 9(10):551. doi: 10.3390/biom9100551.

[31] Thaifa MS, Roshna S, Arya US *et al*., 2017. A review on diabetes mellitus and diabetic neuropathy: A plant based approach. J of Pharmacog and Phytochem. 6(3):506-510.Doi: https://www.phytojournal.com/archives/2017.v6.i3.1264/a.

[32] Nilay D. Solank, Shailesh K Bhavsar, Dharmang T. Pandya. Role of phyto therapy in diabetic neuropathy and neuro degeneration: from pathogenesis to treatment., The Journal of Phyto pharmacology 2018; 7(2): 152-161. DOI:10.31254/phyto.2018.7209.