

Evaluation of *Bacopa monnieri* (Linn) Flowers Extract in Diabetic Neuropathic Pain in Rats

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Abstract: *Diabetes mellitus is a chronic metabolic disorder leading to various complications, including diabetic neuropathy (DN), characterized by nerve damage and neuropathic pain. This study aimed to effect of ethanolic extract of Bacopa monnieri (EEBM) flowers on diabetic neuropathic pain in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in Sprague Dawley rats via STZ injection (60 mg/kg). Diabetic neuropathy was confirmed after STZ induction in negative control group assessing thermal hyperalgesia using the tail flick test and motor coordination deficits using the rota rod apparatus. Diabetic rats were then treated orally with EEBM at low dose (200 mg/kg) and high dose (400 mg/kg), and Metformin (25mg/kg) as a standard control. The effects of EEBM on pain threshold (tail flick latency) and motor coordination (rota rod falling time) were evaluated. Phytochemical screening of EEBM revealed the presence of alkaloids, carbohydrates, cardiac glycosides, tannins, proteins and amino acids, phenolic compounds, flavonoids, anthraquinones, saponins, and terpenoids. The results demonstrated that STZ-induced diabetic rats exhibited significant thermal hyperalgesia and impaired motor coordination compared to normal control rats. Treatment with both low and high doses of EEBM significantly improved tail flick latency and rota rod falling time in diabetic rats compared to the untreated diabetic group. These findings suggest that Bacopa monnieri flowers extract possesses potential analgesic and neuroprotective effects in the context of diabetic neuropathic pain in rats*

Keywords: Bacopa monnieri, Diabetes mellitus, Diabetic Neuropathic Pain, Neuroprotective, Streptozotocin, Tail Flick Apparatus, Rota Rod Apparatus, Phytochemicals

I. INTRODUCTION

Diabetes is a chronic and complex metabolic disorder characterized by high blood sugar levels, affecting millions of people worldwide. It occurs when the body either cannot produce enough insulin, a hormone regulating blood sugar levels, or cannot effectively utilize the insulin produced. This leads to Hyperglycaemia, causing a range of symptoms including increased thirst and urination, fatigue, and blurred vision. If left untreated or poorly managed, diabetes can lead to severe complications such as heart disease, kidney damage, nerve damage, retina and blood vessels. With the global prevalence of diabetes expected to rise, understanding its causes, diagnosis, treatment options, and management strategies is crucial for improving patient outcomes and quality of life. (American Diabetes Association) The International Diabetes Federation (IDF) estimates an overall prevalence of diabetes mellitus. (Uzman Alam et al, 2014)

1.1 Epidemiology:

The disease burden related to diabetes is high and rising in every country, fuelled by the global Prevalence of 382 million people with diabetes in 2013, expected to rise to 592 million by 2035. The aetiological classification of diabetes has now been widely accepted. Type 1 and type 2 diabetes are the two main types, with type 2 diabetes accounting for the majority (>85%) of total diabetes prevalence. Both forms of diabetes can lead to multisystem complications of microvascular endpoints, including retinopathy, nephropathy and neuropathy, and microvascular endpoints including ischaemic heart



disease, stroke and peripheral vascular disease. The premature morbidity, mortality, reduced life expectancy and financial and other costs of diabetes make it an important public health condition (Nita Gandhi et al., 2010)

1.2 Chronic Complications of Diabetes Mellitus:

A. Microvascular:

- Eye disease
- Retinopathy (non-proliferative/proliferative)
- Macular edema
- Neuropathy
- Sensory and motor (mono- and polyneuropathy)
- Autonomic
- Nephropathy

B. Macrovascular:

- Coronary heart disease
- Peripheral arterial disease
- Cerebrovascular disease

C. Other:

- Gastrointestinal (gastroparesis, diarrhea)
- Genitourinary (uropathy/sexual dysfunction)
- Dermatologic Infectious
- Glaucoma
- Hearing loss

1.3 Diabetic Neuropathy:

It is complication of Diabetes mellitus, Diabetic neuropathy can contribute to a number of high-risk complications, ranging from heart rate changes to visual disturbances. Possible complications include losing sensation in the feet (Yvette Brazier, 2019). This can lead to an inability to feel cuts or sores, and infection might occur as a result. Untreated infection in a limb can result in the need for amputation Severe bladder and kidney infections might also occur, causing health problems. To prevent the complications of diabetic peripheral neuropathy, good foot care is essential (Yvette Brazier, 2019). People with the condition should inspect their feet every day for injuries or sores. Smoking also increases the risk of foot problems in people with certain types of diabetic neuropathy. A podiatrist can help with foot care, and a healthcare provider can give advice on quitting smoking (Yvette Brazier, 2019).

1.4 Diabetic Neuropathic Pain:

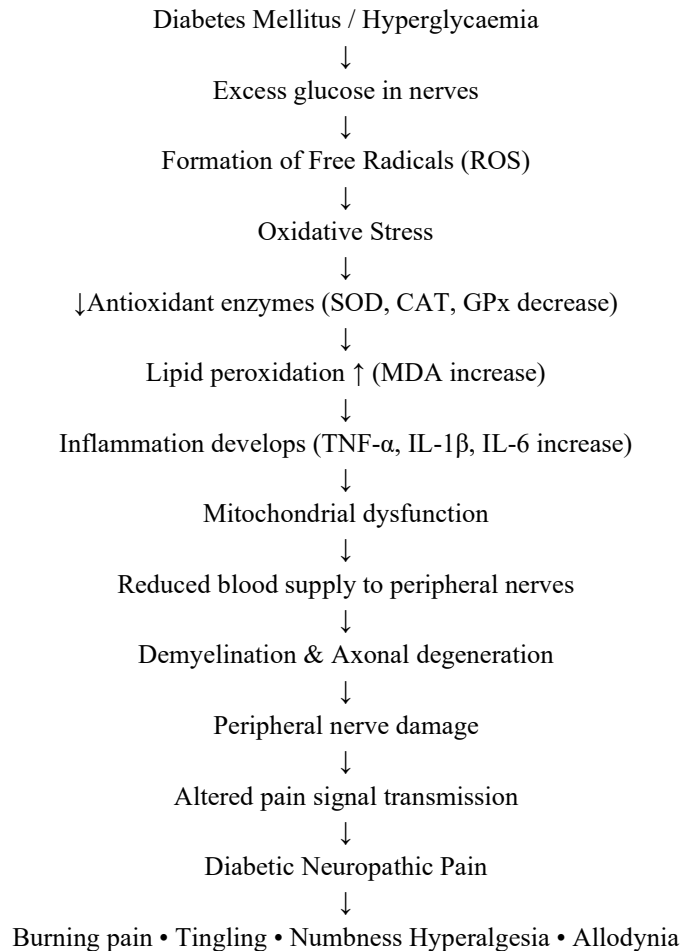
Diabetic neuropathy, a well-known, long-term complication of diabetes, can affect almost half of the diabetic population and is associated with higher morbidity and mortality (Amir Aslam et al., 2014). Diabetic neuropathy encompasses a variety of clinical or subclinical presentations. Painful diabetic neuropathy (PDN) is a common type of diabetic neuropathy and the most common cause of neuropathic pain. The reported prevalence of 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 euro Diab 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), Painful diabetic neuropathy (PDN) symptoms exhibit a symmetrical "stocking and gloves" distribution and are often



associated with nocturnal exacerbation. It can be presented from a mild pins and needle sensation to stabbing, burning, unremitting, or even unpleasant electric shock sensation (Amir Aslam et al., 2014).

1.5 Pathophysiology of Diabetic Neuropathic Pain :

The pathophysiology of diabetic neuropathic pain involves complex mechanisms, including. (Feldman Eva L., Nave K.A., Jensen T.S., et al. 2017)



1.6 Signs and Symptoms:

- ▶ Sensitivity to touch
- ▶ Loss of sense of touch
- ▶ Difficulty with coordination when walking
- ▶ Numbness or pain in your hands or feet
- ▶ Burning sensation in feet, especially at night
- ▶ Muscle weakness or wasting
- ▶ Excessive or decreased sweating
- ▶ Bladder problems, such as incomplete bladder emptying



1.7 Oxidative Stress:

Oxidative stress is an imbalance between the production of Reactive Oxygen Species (ROS/free radicals) and the body's antioxidant defence mechanisms, favouring the accumulation of oxidants. It leads to structural damage of cells, lipids, proteins, mitochondria and DNA, resulting in cellular dysfunction, aging, and various chronic diseases. (Sies H. (1985) Halliwell (2007) & Gutteridge (2015) Normally, small amounts of free radicals are produced during metabolism and are neutralized by antioxidants during metabolism and are neutralized by antioxidants. Oxidative stress = Excess free radicals – Antioxidant defence.

II. MATERIALS AND METHODS

2.1 Materials:

2.1.1 Chemicals and Reagents used during study:

Petroleum Ether, Ethanol, Chloroform, Streptozotocin, Molish's Reagent, Mayer's Reagent, Sulphuric acid, Lead acetate, Ninhydrin Solution, Ammonia Solution, Glucose Diagnostic Kit.

2.1.2 Apparatus and Instruments:

Analgesiometer, Rota-rod Apparatus, Digital weighing balance, Glucometer, Glass wares.

2.1.3 Experimental Animals:

Healthy both sex Sprague Dawley Rats (8 week of age) of weighing 150-250 gm were selected for the study. The animals were housed in polypropylene cages with wire mesh and husk bedding and maintained under standard environmental conditions (temperature 22± 2°C), relative humidity 55-60%, light dark cycle of 12 hours each and rats were fed with standard pellets diet and tap water during course of study. All experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC) with reference no. 650/PO/Re/S-2002/2026/CPCSEA/01 and conducted in accordance with the guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2 Methods:

2.2.1 Plant Material and Extraction:

Bacopa monnieri plant were collected from local area of Akola, Vidarbha region, Maharashtra, (India). The plant material was identified and authenticated by a botanist (Reference No: SSSKRIMV/1868/2026). Flowers of *Bacopa monnieri* plant were collected, dried in shade and coarsely powdered. The powdered flowers were extracted in ethanol by using soxhlet apparatus. The ethanolic extract was concentrated by evaporation at 40°C on Water bath.

2.2.2 Phytochemical Screening:

The ethanolic extract of *Bacopa monnieri* flowers (EEBM) was subjected to qualitative Phytochemical Screening using standard procedures to detect the presence of various secondary metabolites, including alkaloids, carbohydrates, cardiac glycosides, tannins, proteins and amino acids, phenolic compounds, flavonoids, anthraquinones, saponins, and terpenoids.

2.2.3 Induction of Diabetic Neuropathic Pain:

Diabetes was induced in rats by a single intraperitoneal injection of STZ at a dose 60mg/kg to overnight fasted rats. STZ dose was calculated as per body weight of individual animal and it was solubilized with 0.05 M citrate buffer pH 4.5. Negative control rats received an equivalent volume of citrate buffer. blood glucose was measured using glucometer 72 hrs post-STZ injection, and rats with fasting blood glucose levels above 150mg/dl were considered as diabetic. Diabetic Neuropathic pain was confirmed after 4 weeks of induction of Diabetes.



2.2.4 Experimental Groups:

Rats were divided in 5 groups for the study (n=6).

Group-I (Normal Control) = Non diabetic rats treated with normal saline solution 0.9 % (1ml).

Group-II (Negative Control) = Diabetes rats treated by using STZ 60mg/kg i.p

Group-III (Low Dose) = Diabetic rats were treated orally with of EEBM 200mg/kg.

Group-IV (High Dose) = Diabetic rats were treated orally with EEBM 400mg/kg.

Group-V (Standard) = Diabetic rats were treated orally with standard anti-diabetic drugs Metformin 25mg/kg.

2.2.5 Assessment of Diabetic Neuropathic Pain:

A. Thermal Hyperalgesia (Tail Flick Test):

Thermal hyperalgesia was assessed using the tail flick test Rats were gently restrained, and the distal third of the tail was recorded. A cut-off time of 10 seconds was set to prevent tissue damage 41 Measurements were taken at haseline was exposed to a radiant heat source (analgesiometer). The latency of tail withdrawal (flicking) from the heat stimulus day 0, day 3, day 28, and day 42.

B. Motor Coordination (Rota Rod Test):

Motor coordination and muscle strength were assessed using a rota rod apparatus, Rats were placed on a rotating rod (25 rpm), and the time they remained on the rod before falling was recorded (mean falling time, MFT). A shorter fall time compared to the control group indicated reduced muscle strength. Each rat was given three trials with an interval of 30 minutes, and the average falling time was calculated 42 Measurements were taken at baseline day 0, day 3, day 28, and day 42.

2.2.6 Statistical Analysis:

All data were expressed as the mean \pm standard deviation. For statistical Analysis of the rats, were compared by one-way (ANOVA) followed by Dunnett's test, $p < 0.01$ was considered as significant value.

III. RESULTS

3.1 Phytochemical Screening:

Phytochemical Screening of ethanolic extract of *Bacopa monnieri* flowers revealed the presence of a alkaloids, carbohydrates, cardiac glycosides, tannins, proteins and amino acids, phenolic compounds, flavonoids, anthraquinones, saponins, and terpenoids.

Table no. 1 Phytochemical Screening of EEBM

Sr No.	Plant Constituents	Test Performed Reagents	Ethanolic extract
1.	Test for Alkaloids	Mayer's test	+
2.	Test for Carbohydrates	Molish's test	+
3.	Test for Cardiac glycosides	Keller killani test	+
4.	Test for Tannins	Braymer's test	+
5	Test for Proteins/ Amino acids	Ninhydrin test	+
6	Test for Phenolic	Lead acetate test	+



7.	Test for Flavonoids	Ammonia test	+
8.	Test for Anthraquinone	Bontrager test	+
9.	Test for Saponins	Foam test	+
10.	Test for Terpenoid	Salkowski's test	+



Figure No. 1: Phytochemical Screening of ethanolic extract of *Bacopa monnieri* flowers

3.2 Effect of EEBM on Body Weight:

Groups	Body Weight of rat on day 0 (gm)	Body Weight of rat on day 3 (gm)	Body Weight of rat on day 28 (gm)	Body Weight of rat on day 42 (gm)
Normal Control	218±5.67	221.50±12.26	218.50±19.89	227.25±13.94
Negative control	216±20.87 ^{ns}	175.50±6.18 ^{ns}	148.75±6.24 ^{**}	137±6.88 ^{**}
EEBM (200mg/kg)	222±15.15 ^{ns}	174.25±4.11 ^{ns}	172.75±11.12 [@]	179±6.27 ^{@@}
EEBM (400mg/kg)	214±16.32 ^{ns}	171.75±1.71 ^{ns}	198.50±7.19 ^{@@}	195±17.66 ^{@@}
Metformin(25mg/kg)	215±20.89 ^{ns}	191.50±5.80 ^{ns}	210.25±8.38 ^{@@}	213±7.87 ^{@@}



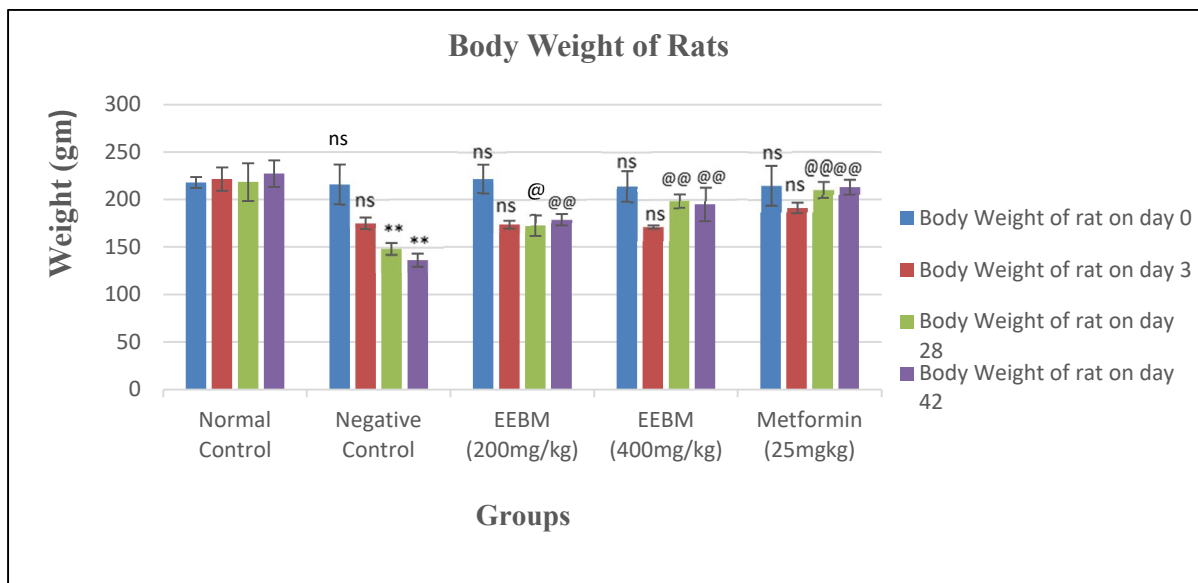


Fig. No. 2: Evaluation of body weight of rats on day 0, 3, 28 and 42.

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

^{ns}p > 0.05, ^{**}p < 0.01 when compared to Control group of rats.

^{ns}p > 0.05, [@]p < 0.05, ^{@@}p < 0.01 when compared to Negative Control group of rats.

Table 7.2 and Figure No. 7.2 show the effect of *Bacopa monnieri* flowers extract on body weight of streptozotocin induced diabetic rats, there was significant decrease (p < 0.01) in the body weight in negative control group compared to normal control group of rats on day 3, 28 and 42. This decrease in the body weight confirms the loss of body weight in diabetes in the rats. After Drug treatment, there was significant improvement (p < 0.01) in low dose group (200 mg / kg) high dose group (400 mg / kg) and Standard dose group compared to negative control groups on day 28 and 42.

3.3 Effect of EEBM on Blood Glucose Level:

Groups	Glucose level on day 0 (mg/dl)	Glucose level on day 3(mg/dl)	Glucose level on day 28 (mg/dl)	Glucose level on day 42 (mg/dl)
Positive Control	100.14±5.24	99.07±2.75	101.54±4.43	100.55±2.27
Negative control	98.56±5.02 ^{ns}	186.50±4.41 ^{ns}	221.82±4.41 ^{**}	240.23±6.99 ^{**}
EEBM (200mg/kg)	102.63±5.01 ^{ns}	189.87±7.24 ^{ns}	189.6±5.87 ^{@@}	179.09±9.15 ^{@@}
EEBM (400mg/kg)	102.78±3.62 ^{ns}	187.15±5.56 ^{ns}	168.11±7.86 ^{@@}	156.66±9.32 ^{@@}
Metformin(25mg/kg)	98.66±4.32 ^{ns}	166.30±3.21 ^{ns}	158.80±9.14 ^{@@}	149.89±10.31 ^{@@}



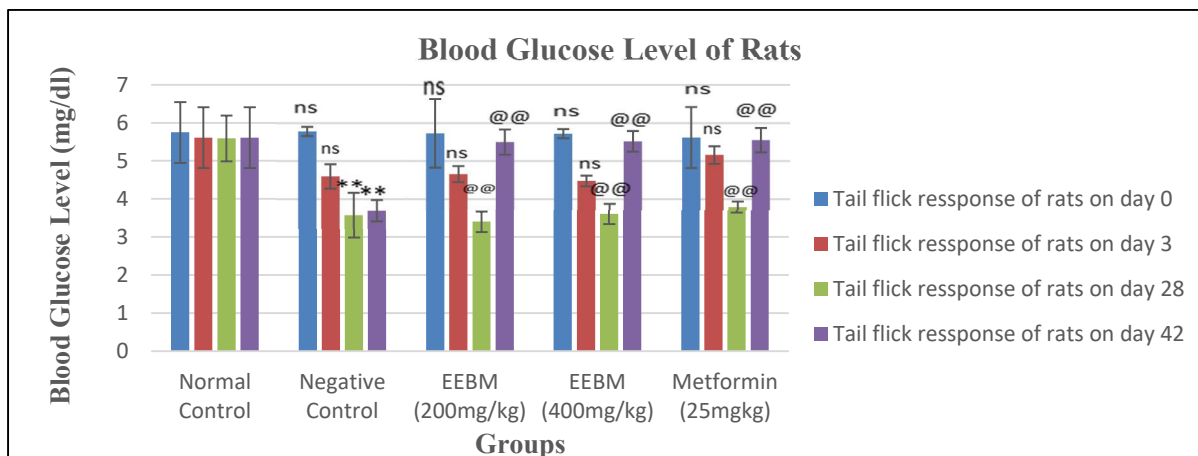


Figure No.3: Estimation of blood glucose level of rats on day 0, 3, 28 and 42.

The results were expressed as Mean \pm SD (n = 6).

^{ns} p > 0.05, ^{**} p < 0.01 when compared to Control group of rats.

^{ns} p > 0.05, ^{@@} p < 0.01 when compared to Negative Control group of rats.

Table No. 7.3 and Figure No. 7.3 shows the effect of *Bacopa monnieri* flowers extract on blood glucose levels of streptozotocin induced diabetic rats, there was significant increase (p < 0.01) in the blood glucose levels in negative control group compared to normal control group of rats on day 3, 28 and 42. This increase in the Blood glucose levels confirms the diabetes in the rats. After drug treatment there was significant decrease (p > 0.01) in the Blood glucose level in low dose group (200 mg / kg) high dose group (400 mg/ kg) Standard dose group compared to negative control on day 28 and 42.

3.4 Effect of EEBM on Tail Flick Test:

Groups	Tail Flick Response of Rats on day 0 (in sec)	Tail Flick Response of Rats on day 3 (in sec)	Tail Flick Response of Rats on day 28 (in sec)	Tail Flick Response of Rats on day 42 (in sec)
Positive Control	5.75 \pm 0.8	5.61 \pm 0.8	5.59 \pm 0.6	5.61 \pm 0.8
Negative control	5.78 \pm 0.12 ^{ns}	4.60 \pm 0.32 ^{ns}	3.58 \pm 0.59 ^{**}	3.70 \pm 0.28 ^{**}
EEBM (200mg/kg)	5.73 \pm 0.9 ^{ns}	4.66 \pm 0.21 ^{ns}	4.40 \pm 0.27 ^{@@}	5.50 \pm 0.33 ^{@@}
EEBM (400mg/kg)	5.72 \pm 0.12 ^{ns}	4.48 \pm 0.14 ^{ns}	4.61 \pm 0.27 ^{@@}	5.52 \pm 0.27 ^{@@}
Metformin (25mg/kg)	5.62 \pm 0.8 ^{ns}	5.16 \pm 0.23 ^{ns}	4.79 \pm 0.15 ^{@@}	5. \pm 0.32 ^{@@}



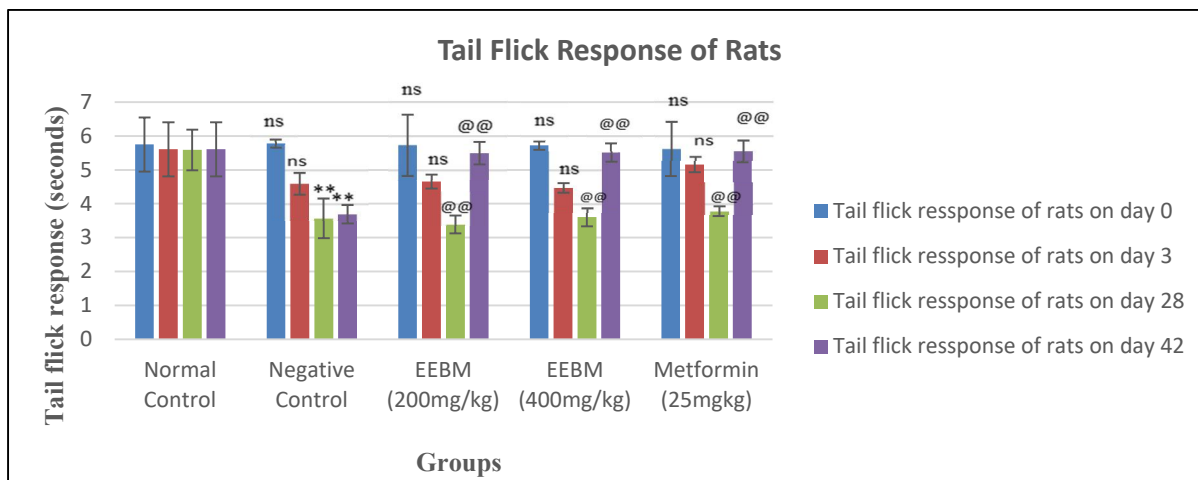


Figure No. 4: Tail Flick response of rats on day 0, 3, 28 and 42.

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

^{ns}p > 0.05, ^{**}p < 0.01 when compared to Control group of rats.

^{ns}p > 0.05, ^{@@}p < 0.01, when compared to Negative Control group of rats

Table No. 7.4 and Figure No. 7.4 reveals the effect of *Bacopa monnieri* flowers extract on tail flick pain sensation effect of streptozotocin induced diabetic rats, 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 day 3, 28 and 42. This decrease in the analgesic effect confirms the hyperalgesia in rats. After drug treatment, there was Significant improvement (p<0.01) in tail flick response in low dose group (200mg / kg) high dose group (400mg / kg) and Standard dose group compared to negative control groups on day 28 and 42.

3.5 Effect of EEBM on Muscle Coordination:

Groups	Mean falling time of rat on day 0 (in sec)	Mean falling time of rat on day 3 (in sec)	Mean falling time of rat on day 28 (in sec)	Mean falling time of rat on day 42 (in sec)
Positive Control	58.82±3.74	56.06±2.63	59.89± 3.02	53.62± 2.67
Negative control	55.12±3.57 ^{ns}	39.98 ± 3.23 ^{ns}	31.06 ±3.52 ^{**}	28.01± 4.94 ^{**}
EEBM (200mg/kg)	55.57±4.00 ^{ns}	40.08 ± 2.15 ^{ns}	39.35 ±2.26 ^{@@}	45.33± 2.95 ^{@@}
EEBM (400mg/kg)	60.48±3.46 ^{ns}	39.35± 2.53 ^{ns}	48.59 ±2.61 ^{@@}	51.06 ±4.13 ^{@@}
Metformin (25mg/kg)	59.80±3.70 ^{ns}	51.36 ±3.46 ^{ns}	57.33 ±2.51 ^{@@}	57.58 ±2.86 ^{@@}



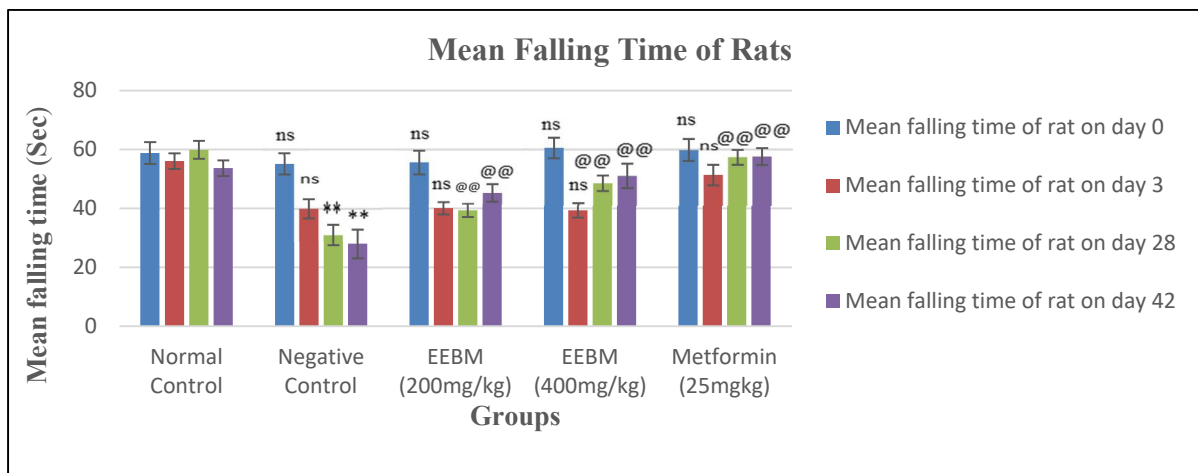


Figure 7.5: Muscle co-ordination response of rats on rota rod apparatus on day 0, 3, 28 and 42.

The results were expressed as Mean \pm SD (n = 6).

^{ns} p > 0.05, *p < 0.01 when compared to Control group of rats.

^{ns} p > 0.05, @@p > 0.01, when compared to Negative Control group of rats.

Table No. 7.5 and Figure No. 7.5 reflect the effect of *Bacopa monnieri* flowers extract on muscle strength of streptozotocin induced diabetic rats, There was significant decrease (p < 0.01) in the muscle strength of rats in negative control group compared to normal control group of rats on day 3, 28 and 42. This decrease in the muscle coordination confirms Neuropathy, After drug treatment there was significant improvement (p < 0.01) in the muscle Co-ordination in low dose group (200 mg / kg) high dose group (400 mg / kg) and Standard dose group compared to negative control on day 28 and 42.

3.6 Oxidative Stress Parameters in Blood:

3.6.1. Malondialdehyde (MDA nmol/mL):

Groups	MDA on day 0	MDA on day 3	MDA on day 28	MDA on day 42
Normal Control	3.42 \pm 0.35	3.48 \pm 0.31	3.51 \pm 0.29	3.46 \pm 0.33
Negative control	3.40 \pm 0.38	8.22 \pm 0.84 ^{ns}	8.94 \pm 0.91 ^{**}	9.35 \pm 0.88 ^{**}
EEBM (200mg/kg)	3.45 \pm 0.32	8.15 \pm 0.79 ^{ns}	5.92 \pm 0.54 ^{@@}	4.55 \pm 0.42 ^{@@}
EEBM (400mg/kg)	3.39 \pm 0.41	8.20 \pm 0.81 ^{ns}	4.81 \pm 0.46 ^{@@}	3.88 \pm 0.36 ^{@@}
Metformin (25mg/kg)	3.44 \pm 0.30	8.25 \pm 0.75 ^{ns}	4.65 \pm 0.40 ^{@@}	3.72 \pm 0.31 ^{@@}



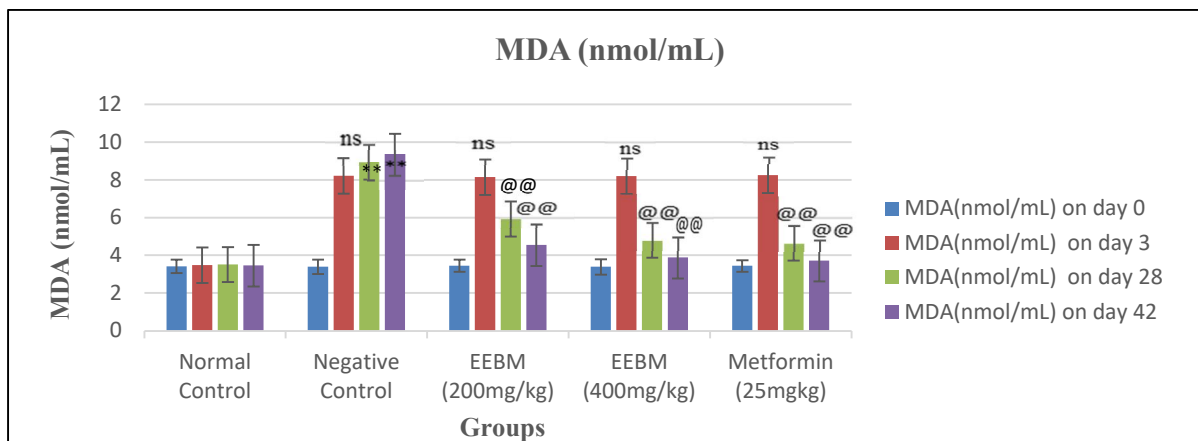


Figure 7.6.1: Effect of Bacopa monnieri on Lipid peroxidation Activity in Rats on day 0,3,28,42.

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

^{ns}p > 0.05 ^{**}p < 0.01. ^{***}p < 0.01 when compared to positive control groups of rats.

^{ns}p > 0.05, @p < 0.01, @@@p < 0.01 when compared to negative control groups of rats.

Table 7.6.1 and figure 7.6.1 reflects the effect of STZ on Lipid peroxidation activity (MDA) in rats on 0,3,28,42 day of STZ induction. After the confirmation of Diabetic Neuropathy, rats were treated with ethanolic extract of *Bacopa monnieri* for six weeks. After drug treatment there was significant improvement (p<0.01) in the Lipid peroxidation activity in STZ + Bm flowers extract (200 mg/kg) and STZ + Bm flowers extract (400 mg/kg) treated group compared to negative control group.

3.6.2: Superoxide Dismutase Activity (SOD U/mg Protein):

Groups	SOD on day 0	SOD on day 3	SOD on day 28	SOD on day 42
Normal Control	25.40±2.10	25.15±1.95	24.85±2.20	25.10±1.85
Negative control	25.12 ±2.30	11.10±1.45 ^{ns}	9.45±1.15 ^{**}	8.80±1.05 ^{**}
EEBM (200mg/kg)	24.25±1.90	10.95±1.30 ^{ns}	16.80±1.65 ^{@@}	20.90±1.95 ^{@@}
EEBM (400mg/kg)	25.25±2.05	11.20±1.50 ^{ns}	19.95±1.80 ^{@@}	23.95±2.10 ^{@@}
Metformin (25mg/kg)	25.30±1.98	11.05±1.35 ^{ns}	20.45±1.70 ^{@@}	24.15±1.90 ^{@@}

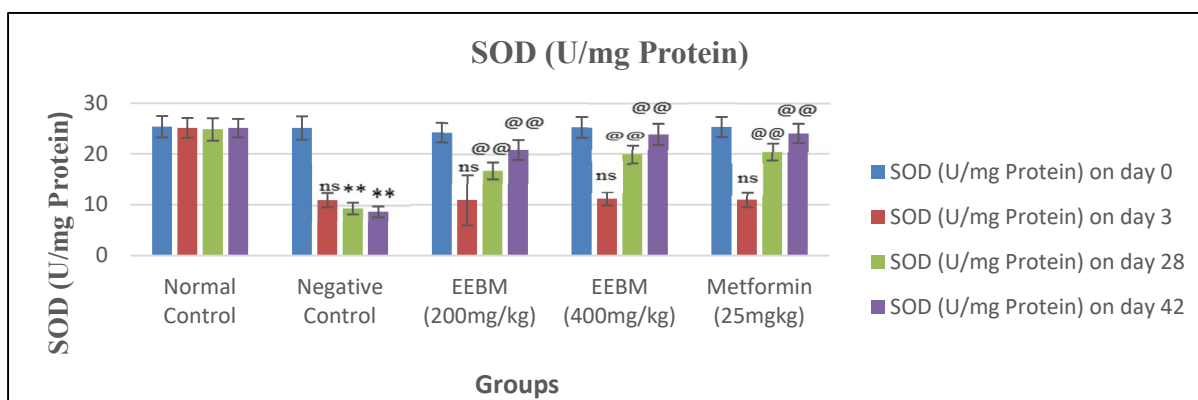


Figure 7.6.2: Effect of Bacopa monnieri on Superoxide Dismutase Activity in Rats on day 0,3,28,42.



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

^{ns}p > 0.05 ** p < 0.01. ***p < 0.01 when compared to positive control groups of rats

^{ns}p > 0.05, @@p < 0.01, @@@p < 0.01 when compared to negative control groups of rats.

Table 7.6.2 and figure 7.6 reflects the effect of STZ on Superoxide Dismutase activity (SOD) in rats on 0,3,28,42 day of STZ induction. After the confirmation of Diabetic Neuropathy, rats were treated with ethanolic extract of *Bacopa monnieri* for six weeks. After drug treatment there was significant improvement (p<0.01) in the Lipid peroxidation activity in STZ + Bm flowers extract (200 mg/kg) and STZ + Bm flowers extract (400 mg/kg) treated group compared to negative control group.

3.6.3 Glutathione Peroxidase (GPx U/mg Protein):

Groups	GPx on day 0	GPx on day 3	GPx on day 28	GPx on day 42
Normal Control	14.25±1.15	14.10±1.05	14.38±1.22	14.18±1.10
Negative control	14.12±1.20	6.95±0.78 ^{ns}	5.12±0.65 ^{**}	4.45±0.58 ^{**}
EEBM (200mg/kg)	14.30±1.08	7.05±0.82 ^{ns}	9.15±0.92 ^{@@}	11.85±1.02 ^{@@}
EEBM (400mg/kg)	13.98±1.25	7.12±0.76 ^{ns}	11.20±1.05 ^{@@}	13.10±1.12 ^{@@}
Metformin (25mg/kg)	14.15±1.10	7.08±0.74 ^{ns}	11.85±0.98 ^{@@}	13.45±1.05 ^{@@}

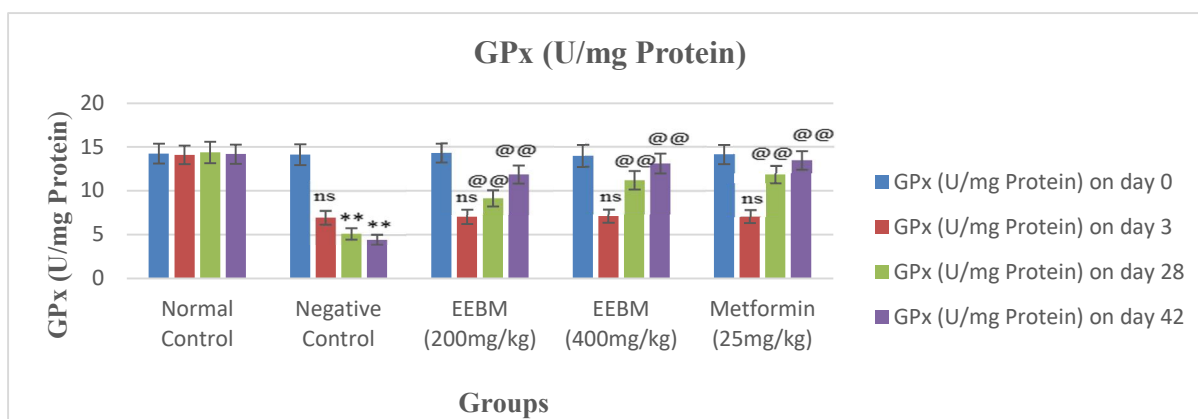


Figure 7.6.3: Effect of Bacopa monnieri on Glutathione Peroxidase Activity in Rats on day 0,3,28,42.

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

^{ns}p > 0.05 ** p < 0.01. ***p < 0.01 when compared to positive control groups of rats

^{ns}p > 0.05, @@p < 0.01, @@@p < 0.01 when compared to negative control groups of rats.

Table 7.6.3 and figure 7.6.3 reflects the effect of STZ on Glutathione Peroxidase activity (GPx) in rats on 0,3,28,42 day of STZ induction. After the confirmation of Diabetic Neuropathy, rats were treated with ethanolic extract of *Bacopa monnieri* for six weeks. After drug treatment there was significant improvement (p<0.01) in the Lipid peroxidation activity in STZ + BM flowers extract (200 mg/kg) and STZ + BM flowers extract (400 mg/kg) treated group compared to negative control group.



3.6.4 Catalase (CAT U/ mg Protein):

Groups	CAT on day 0	CAT on day 3	CAT on day 28	CAT on day 42
Normal Control	46.20±3.80	45.85±4.10	46.50±3.95	45.90±4.20
Negative control	45.90±4.15	23.80±3.20 ^{ns}	18.10±2.35 ^{**}	16.30±2.10 ^{**}
EEBM (200mg/kg)	46.10±3.90	24.10±3.05 ^{ns}	31.60±3.25 ^{@@}	39.40±3.70 ^{@@}
EEBM (400mg/kg)	46.55±4.25	24.55±3.10 ^{ns}	36.90±3.60 ^{@@}	43.15±3.90 ^{@@}
Metformin (25mg/kg)	46.05±4.00	24.90±2.95 ^{ns}	37.40±3.15 ^{@@}	43.80±3.55 ^{@@}

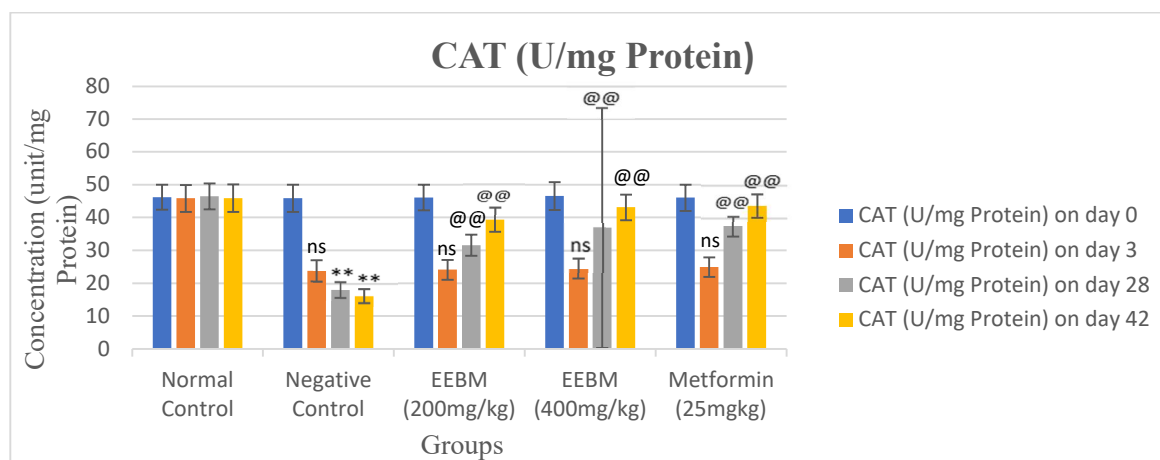


Figure 7.6.4: Effect of Bacopa monnieri on Catalase Activity in Rats on day 0,3,28,42.

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

^{ns}p > 0.05 ^{**}p < 0.01. ^{***}p < 0.01 when compared to positive control groups of rats

^{ns}p > 0.05, ^{@@}p < 0.01, ^{@@@}p < 0.01 when compared to negative control groups of rats.

Table 7.6.4 and figure 7.6.4 reflects the effect of STZ on Catalase activity (SOD) in rats on 0,3,28,42 day of STZ induction. After the confirmation of Diabetic Neuropathy, rats were treated with ethanolic extract of *Bacopa monnieri* for six weeks. After drug treatment there was significant improvement (p<0.01) in the Lipid peroxidation activity in STZ + Bm flowers extract (200 mg/kg) and STZ + Bm flowers extract (400 mg/kg) treated group compared to negative control group.

IV. DISCUSSION

The present study demonstrated that streptozotocin (STZ) successfully induced diabetic neuropathy in rats, as evidenced by hyperglycaemia, weight loss, increased pain sensitivity, impaired motor coordination, and oxidative stress. STZ-treated diabetic rats showed significant elevation in blood glucose levels and reduction in body weight compared with normal control animals, confirming successful induction of diabetes. These findings are consistent with previous reports that STZ causes selective destruction of pancreatic β-cells leading to insulin deficiency and hyperglycaemia (Szkudelski, 2001). Treatment with *Bacopa monnieri* flower extract significantly improved body weight and reduced blood glucose levels, indicating possible antihyperglycemic activity.

Behavioural assessments revealed significant development of diabetic neuropathic pain in diabetic control rats. Tail flick latency was significantly reduced, indicating thermal hyperalgesia due to peripheral nerve injury and altered nociceptive transmission caused by chronic hyperglycaemia and oxidative stress. Similar findings have been reported in



experimental diabetic neuropathy studies (Calcutt et al., 2009; Vincent et al., 2011). Administration of *Bacopa monnieri* flower extract significantly increased tail flick latency, suggesting attenuation of neuropathic pain and neuroprotective activity. Rotarod performance also showed reduced mean falling time in diabetic rats, indicating impaired motor coordination and neuromuscular dysfunction, whereas treatment with the extract significantly improved motor performance.

Oxidative stress plays a crucial role in the pathogenesis of diabetic neuropathy. Diabetic control rats exhibited significantly increased malondialdehyde (MDA) levels along with reduced antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), indicating enhanced lipid peroxidation and weakened antioxidant defence. These findings are in agreement with previous studies showing that chronic hyperglycaemia increases reactive oxygen species production and oxidative damage in diabetic neuropathy (Feldman et al., 2019; Vincent et al., 2011). Treatment with *Bacopa monnieri* flower extract significantly reduced MDA levels and restored SOD, GPx, and CAT activities, suggesting potent antioxidant and free radical scavenging properties.

Overall, the findings suggest that *Bacopa monnieri* flower extract possesses significant antidiabetic, antioxidant, antinociceptive, and neuroprotective activities against STZ-induced diabetic neuropathy in rats. The beneficial effects may be attributed to phytoconstituents such as bacosides, flavonoids, and phenolic compounds, which help reduce oxidative stress and improve nerve function.

V. CONCLUSION

The present study concludes that the ethanolic extract of *Bacopa monnieri* flowers possesses significant antidiabetic, antioxidant, analgesic, and neuroprotective activity against streptozotocin-induced diabetic neuropathic pain in rats. Treatment with the extract significantly reduced elevated blood glucose levels and improved body weight in diabetic animals. The extract at doses of 200 mg/kg and 400 mg/kg showed marked improvement in analgesic response and motor coordination activity, indicating protection against neuropathic pain and nerve damage.

The study also demonstrated significant improvement in biochemical parameters, including reduction in serum glucose, triglycerides, cholesterol, and lipid peroxidation levels, along with restoration of antioxidant enzymes such as SOD, CAT, and GPx. These findings suggest that the beneficial effects of the extract may be due to the presence of bioactive phytoconstituents with antioxidant and free radical scavenging properties. Overall, the ethanolic extract of *Bacopa monnieri* flowers showed promising therapeutic potential in ameliorating diabetic neuropathy and oxidative stress-associated complications of diabetes.

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