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Research Article

Evaluation of EETPAE from *Tridax procumbens* and *Andrographis echinoides* in The Management of Diabetic Neuropathy

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ABSTRACT

Diabetic neuropathy, a prevalent and debilitating complication of diabetes mellitus, affects up to 50% of individuals over the course of the disease. Its prevalence is influenced by multiple factors, including the type and duration of diabetes, glycemic control, age, lifestyle factors, and genetic predisposition. Global disparities exist, with higher rates reported in developed countries due to differences in healthcare access and disease management. The World Health Organization recognizes diabetic neuropathy as a significant public health concern, given its association with increased morbidity, healthcare utilization, and risk of severe complications such as foot ulcers and cardiovascular disease. The current study investigates the therapeutic potential of EETPAE (Ethanollic Extract of *Tridax procumbens* and *Andrographis echinoides*) in mitigating symptoms of diabetic neuropathy. EETPAE exhibited significant analgesic, antioxidant, and metabolic regulatory properties. In experimental pain models, the extract improved pain thresholds and reduced inflammation, while biochemical assays revealed restoration of antioxidant enzymes (CAT, SOD, GSH) and reduction of lipid markers such as free fatty acids and total cholesterol. These effects, especially at the high dose (500 mg/kg), were comparable to standard drugs, suggesting the extract's effectiveness in managing neuropathic pain and metabolic dysregulation. Overall, EETPAE demonstrates promising potential as a natural therapeutic agent for addressing key aspects of diabetic neuropathy, including pain, oxidative stress, and lipid imbalance. Further studies are required to isolate the active compounds and elucidate the molecular mechanisms involved.

INTRODUCTION

Long standing diabetes mellitus leads to multiple organ damage and it is associated with an increased prevalence of micro vascular disease

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(nephropathy, neuropathy & retinopathy) and macro vascular diseases (peripheral vascular disease, ischemic heart disease & stroke). Poor glycemic control, a factor that has been observed in the Indian population with diabetes put them at risk of complication including neuropathy-24.6%, cardiovascular disease - 23.6%, kidney problem - 21.1%, retinopathy- 16.6% and foot ulcer- 5.5% 4. Globally diabetic neuropathy affects approximately 132 million people as of 2010 (1.9% of the population). Diabetes is the leading known cause of neuropathy in developed countries, and neuropathy is the most common complication and greatest source of morbidity and mortality in diabetes.

It is believed that neuropathy affects approximately 25% of individuals diagnosed with diabetes. Diabetic neuropathy is involved in 50–75% of amputations that are not caused by trauma. The primary cause of diabetic neuropathy is high blood sugar. In the DCDT (Diabetes Control and Complications Trial, 1995) study, the yearly occurrence of neuropathy was 2%, but it significantly decreased to 0.56% with intensive treatment of type 1 diabetics. The advancement of neuropathy is influenced by the level of blood

sugar control in both type 1 and type 2 diabetes. The length of time a person has diabetes, their age, smoking habits, blood pressure, height, and high levels of lipids in the blood are all factors that increase the risk of developing diabetic neuropathy. Diabetic neuropathy is a condition that occurs when nerves are harmed as a result of prolonged high blood sugar levels or hyperglycemia, which is a complication of diabetes. Diabetic neuropathy can impact various body parts, such as the legs, feet, bladder, heart, gastrointestinal system, and reproductive system.

Diabetic neuropathy typically progresses gradually over several months, caused by long-term elevated blood sugar levels that harm the nerves in the body. Diabetic neuropathy can manifest as a range of sensations, such as pain, numbness, tingling, or prickling, which typically starts in the feet. In advanced stages of diabetic neuropathy, the hands can also be impacted. In certain instances of diabetic neuropathy, the abnormal sensations can also affect the arms, legs, and torso. Several medications are used to relieve nerve pain, but they don't work for everyone and most have side effects that must be weighed against the benefits they offer.

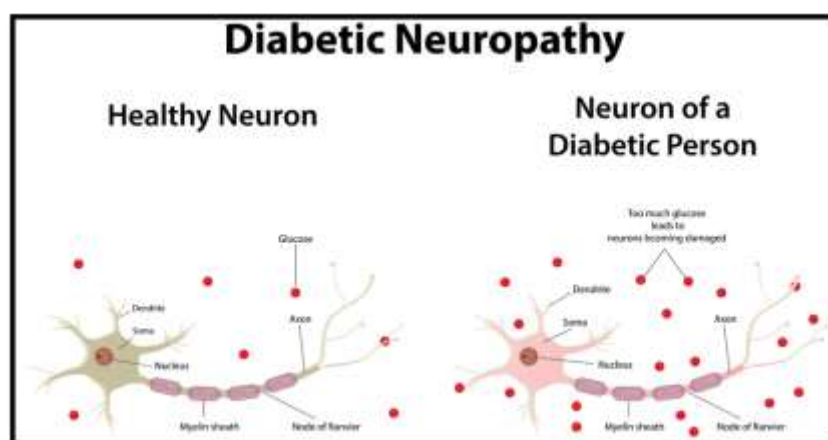


Fig: 1 Diabetic Neuropathy

2. PLANT PROFILE:

2.1 TRIDAX PROCUMBENS L:

Tridax procumbens, commonly called coat buttons or tridax daisy, maybe a species of flowering plant



within the daisy family. It's best called a widespread weed and pest plant. *Tridax procumbens* is traditionally used ayurvedic medicine for hair growth, liver disorder, diabetic and is sometimes dispensed in situ of Bhringraj. The current study was aimed to assess the efficacy of *Tridax procumbens*'s use in hair growth-promoting activity.

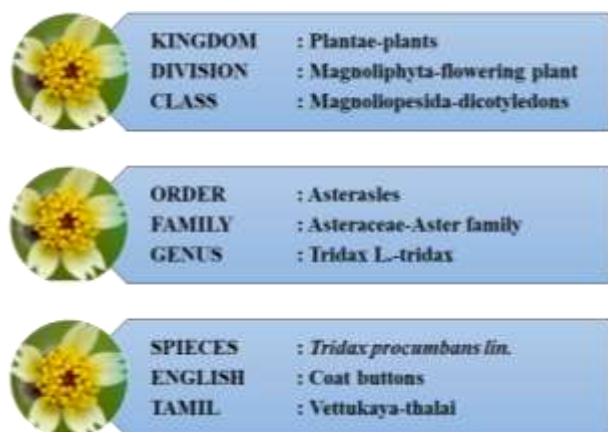


Fig: 2 Plant Profile of *Tridax Procumbens* L

2.2 ANDROGRAPHIS ECHIOIDES NEES:

Scientific classification of plant can be defined as “The arrangement of entities of that plant” in a hierarchical series of nested classes, in which similar or related classes at one hierarchical level are combined comprehensively into more inclusive classes at the next higher level. The scientific classification of *Andrographis echiodides* is as follows.

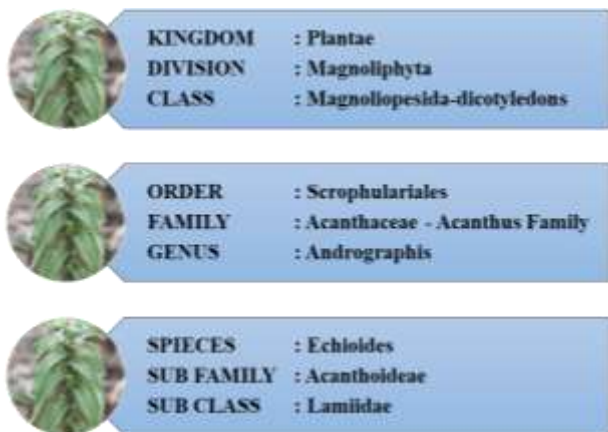


Fig: 3 Plant Profile of *Andrographis echiodides*

3. MATERIALS AND METHODS:

3.1 COLLECTION OF SELECTED HERBS:

Tridax procumbens L, *Andrographis echiodides* L collected from in around Thiruvannamalai district, Tamilnadu. Collected herbs were authenticated by SIDDHA CENTRAL RESEARCH INSTITUTE, Arumbakkam, Chennai.

- **AUTHENTICATION CERTIFICATE FOR 1272.29042518-19**
- **Form No: PCOG002-ACF**
- **Date: 21-05-2025**
- ***Tridax procumbens* L (Code – T29042518P) (Part – Whole Plant)**
- ***Andrographis echiodides* (L.) Nees (Code – A29042519E) (Part – Whole Plant)**

3.2 EXTRACTION:

The whole plant of *Tridax procumbens* L, *Andrographis echiodides* L, were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No:40 and stored in an airtight container for further use.

Preparation Of Ethanolic Extract: Collect dried plant of *Tridax procumbens* L, *Andrographis echiodides* L, were cleaned with water & shade dried until a constant weight was obtained & subsequently powdered & sieved mesh no 40. Powdered material 5kg was extracted with of water at 50 degrees in soxhlet apparatus 1L for 72hr. dark brown semi – solid residues. 525g was obtained by evaporating the aqueous extract under reduced pressure.

3.3 PHYTOCHEMICAL TEST:

The aqueous extracts of *Tridax procumbens* L, & *Andrographis echiodides* L, were subjected to the following preliminary phytochemical analysis

3.4 ACUTE TOXICITY STUDIES:

The procedure was followed by using OECD guidelines (Organization for Economic Co-operation and Development) 423 (acute toxic class method). The acute toxicity study is a Step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or Morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on The acute toxicity of the substances. This procedure results in the use of a specified number of Animals while allowing for acceptable data-based scientific conclusion. Albino mice weighed 25-30gms were used for the study. Body weights of mice before and after administration were observed for morbidity and Mortality. Any changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behaviour pattern were observed and also sign of Tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted.

3.5 EXPERIMENTAL DESIGN:

After six weeks of verifying hyperglycemia in mice, the diabetic-induced mice were assessed for peripheral diabetic neuropathy using von Frey filaments (a tactile allodynia test) and the hot plate test at 55°C (a thermal hyperalgesia test). Once neuropathy development was confirmed, the diabetic mice were distributed into several groups, designated as Group II through, each comprising 5 animals (n=5).

- **Group-I** (Control group) (n=5) receives normal saline (0.9% NaCl)
- **Group-II** (Negative Control group) (n=5) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + receives normal saline for 30 days after confirming diabetic neuropathy.

- **Group-III** (Standard group) (n=5) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Duloxetine (30 mg/kg/day) for 30 days after confirming diabetic neuropathy.
- **Group-IV** (Low dose group) (n=5) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Low dose (250 mg/kg/day) EETPAE (Ethanollic Extract of *Tridax procumbens* & *Andrographis echinoides*) for 30 days after confirming diabetic neuropathy.
- **Group-V** (High dose group) (n=5) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Low dose (500 mg/kg/day) EETPAE (Ethanollic Extract of *Tridax procumbens* & *Andrographis echinoides*) for 30 days after confirming diabetic neuropathy.

3.6 PHARMACOLOGICAL ACTIVITY:

- Hot Plate Test
- Cold Plate Test
- Von Frey Filament Test
- Formalin Test
- Superoxide Dismutase (SOD) Activity
- Catalase (CAT)
- Glutathione Reductase (GSH)
- Blood Glucose Level
- Free Fatty acid
- Total Cholesterol Level

4. RESULTS AND DISCUSSION:

4.1 EXTRACTION:

Table: 1 Extraction

Sr. No	Content	EETPAE
1	Solvent	Ethanol 90%
2	Colour	Light Green
3	Physical Nature	Semi liquids
4	Yield w/w	9.15





Fig: 4 Extraction Of EETPAE

4.2 PHYTO-CHEMICAL TEST:

Table: 2 Phyto-Chemical Test

Sr. No.	Phytochemicals	Presence or Absence
1	Alkaloids	Presence
2	Flavonoids	Presence
3	Tri-terpenoids	Presence
4	Glycosides	Presence
5	Phenolic compounds	Presence
6	Tannins	Presence
7	Anthocyanins	Absence
8	Sterols	Presence
9	Saponins	Absence
10	Carbohydrate	Absence
11	Amino acids	Absence
12	Protein	Absence



Flavonoids



Tannins



Phenol



Glycosides



Terpenoids



Alkaloids

Fig: 5 Phyto – Chemical Test

4.3 ACUTE ORAL TOXICITY STUDIES OF EETPAE (OECD 423 GUIDELINES):

Table: 3 Acute Oral Toxicity Studies

Sr. No.	Treatment Group	Dose	Weight of Animal (In Grams)		Signs of Toxicity	Onset of Toxicity	Reversible/ Irreversible	Duration
			Before Test	After Test				
1	EETPAE	2g/Kg	24	27	No signs of toxicity	NIL	NIL	14 DAYS

2	EETPAE	2g/Kg	26	28	No signs of toxicity	NIL	NIL	14 DAYS
3	EETPAE	2g/Kg	25	25	No signs of toxicity	NIL	NIL	14 DAYS

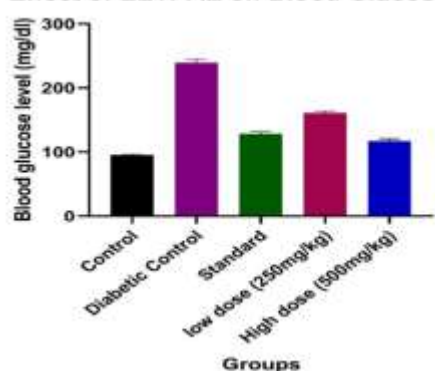
4.4 PHARMACOLOGICAL ACTIVITY:

4.4.1 Assessment of Blood Glucose level & Thermal Hyperalgesia:

Table: 4 Assessment of Blood Glucose level & Thermal Hyperalgesia

Sr. No	Groups	Blood glucose level (mg/dl)	With drawl latency (in sec.)
1	Control	95.22 ± 0.4970	16.88 ± 0.275
2	Negative Control	239.3 ± 2.625 a ****	8.476 ± 0.182 a ****
3	Positive Control	128.1 ± 1.632 a **** b ****	16.92 ± 0.180 a ^{ns} b ****
4	Low dose- 250 mg/kg	160.9 ± 1.317 a **** b **** c ****	10.94 ± 0.242 a **** b **** c ****
5	High dose- 500 mg/kg	116.9 ± 1.508 a **** b **** c ****	13.39 ± 0.147 a **** b **** c ****

Effect of EETPAE on Blood Glucose level



Effect of EETPAE on Hotplate Test

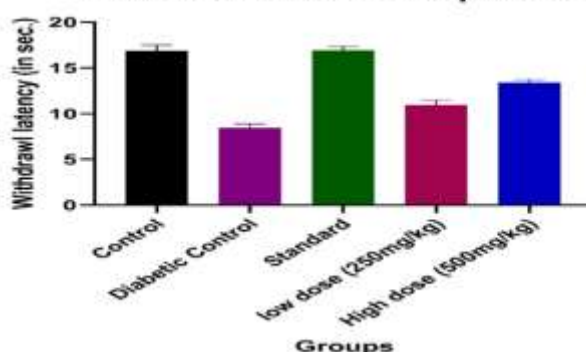


Fig: 6 Assessment of Blood Glucose level & Thermal Hyperalgesia

4.4.2 Effect of EETPAE on Cold Plate Test & Mechanical Hyperalgesia:

Table: 5 Effect of EETPAE on Cold Plate Test & Mechanical Hyperalgesia

Sr. No.	Groups	Withdrawal Latency (In Sec.)	Escape latency (in sec.)
1	Control	14.41 ± 0.083	287.9 ± 0.515
2	Negative Control	3.586 ± 0.128 a ****	94.49 ± 0.327 a ****
3	Positive Control	11.05 ± 0.184 a **** b ****	258.1 ± 0.996 a **** b ****
4	Low dose- 250 mg/kg	8.470 ± 0.141 a **** b **** c ****	139.8 ± 0.650 a **** b **** c ****
5	High dose- 500 mg/kg	10.66 ± 0.112 a **** b **** c ^{ns}	217.9 ± 1.238 a **** b **** c ****

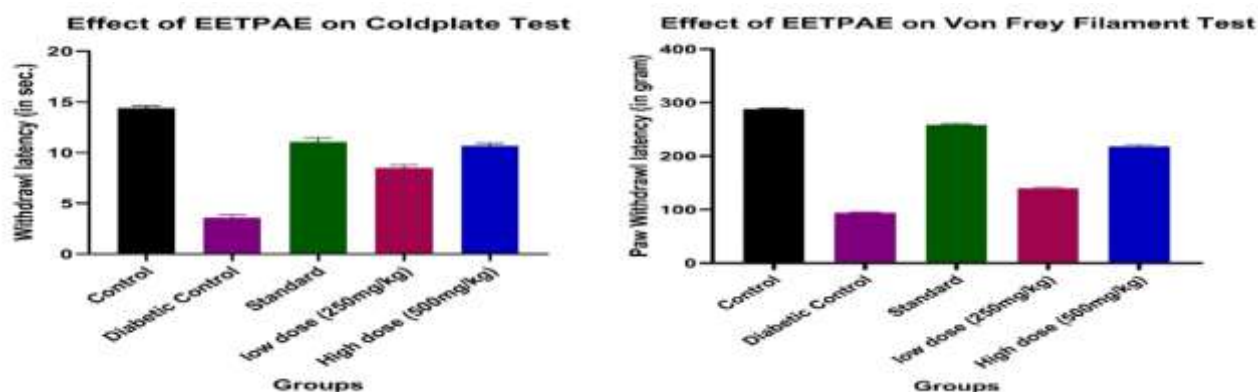


Fig: 7 Effect of EETPAE on Cold Plate Test & Mechanical Hyperalgesia

4.4.3 Effect of EETPAE on Formalin I & II Test:

Table: 6 Effect of EETPAE On Formalin Test- I

Sr. No.	GROUPS	Formalin test- I (Acute Phase)	
		Paw licking (in sec.)	Paw Elevation (in sec.)
1	Control	82.40 ± 0.154	151.2 ± 0.459
2	Negative Control	167.6 ± 0.603 a ****	236.8 ± 1.640 a ****
3	Positive Control	100.7 ± 0.286 a **** b ****	134.9 ± 0.341 a **** b ****
4	Low dose- 250 mg/kg	135.3 ± 0.391 a **** b **** c ****	209.7 ± 0.555 a **** b **** c ****
5	High dose- 500 mg/kg	111.1 ± 0.209 a **** b **** c ****	154.9 ± 0.860 a ^{ns} b **** c ****

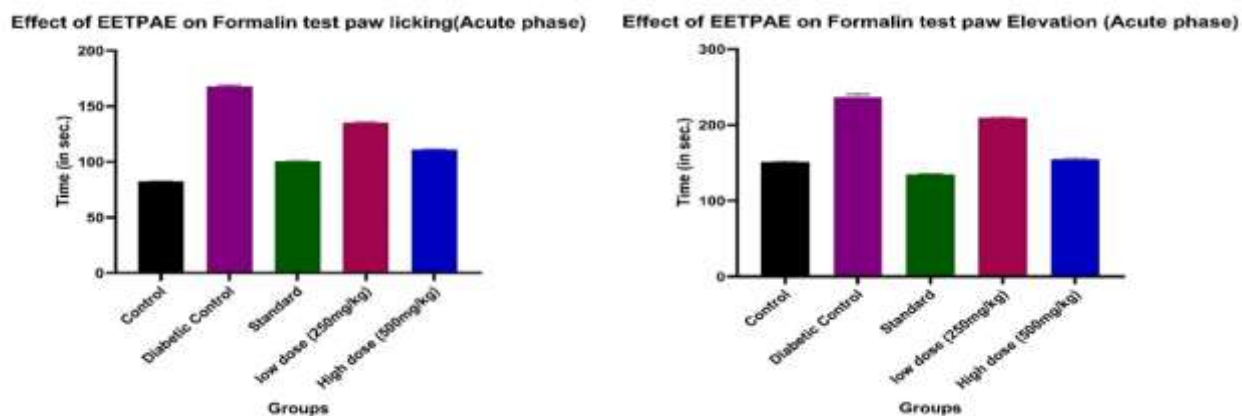


Fig: 8 Effect of EETPAE On Formalin Test- I

Table: 7 Effect of EETPAE On Formalin Test- II

S.NO	GROUPS	Formalin test- II (Delayed Phase)	
		Paw licking (in sec.)	Paw Elevation (in sec.)
1	Control	75.72 ± 0.223	145.0 ± 0.363
2	Negative Control	149.1 ± 0.490 a ****	303.3 ± 0.333 a ****

3	Positive Control	94.81 ± 0.227 a*** b***	129.0 ± 0.450 a*** b***
4	Low dose- 250 mg/kg	134.1 ± 0.191 a*** b*** c***	224.8 ± 0.276 a*** b*** c***
5	High dose- 500 mg/kg	118.0 ± 0.205 a*** b*** c***	162.2 ± 0.351 a*** b*** c***

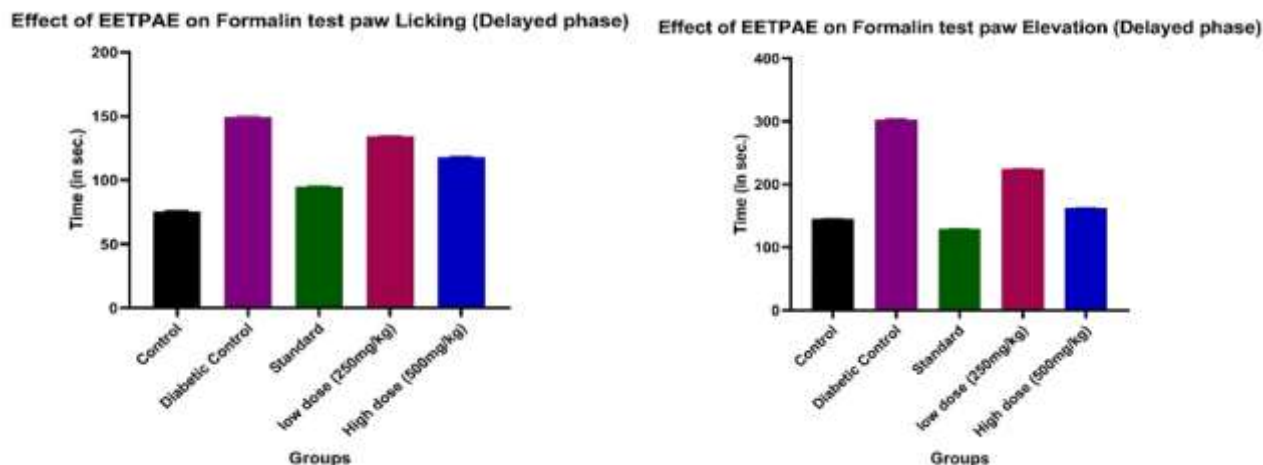
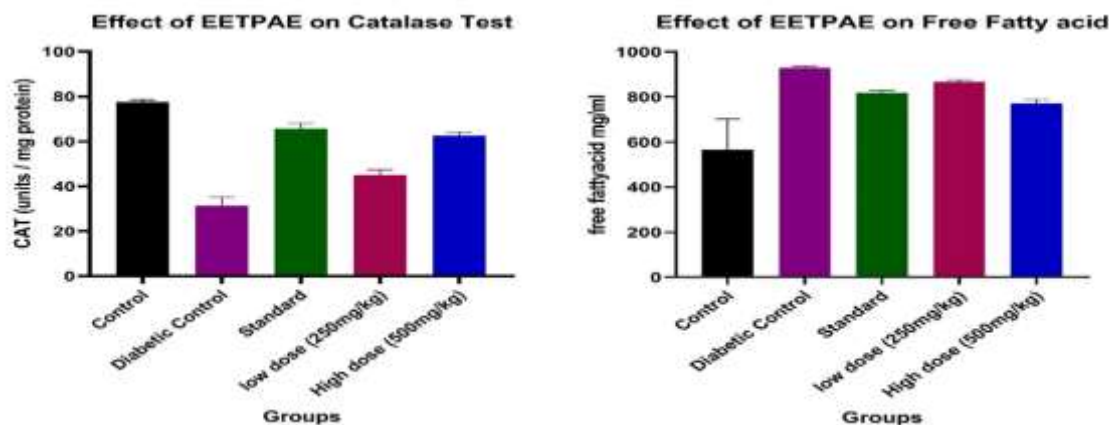


Fig: 9 Effect of EETPAE On Formalin Test- II

4.4.4 ASSESSMENT OF BIOCHEMICAL ANALYSIS:

Table: 8 Assessment of Biochemical Analysis

Sr. No.	Groups	CAT (units / mg protein)	Free fatty acid (mg/ml)	SOD (mg/ml)	Total Cholesterol (mg/ml)	GSH (mg/ml)
1	Control	77.61 ± 0.399	566.0 ± 1.034	11.56 ± 0.107	34.03 ± 0.554	82.33
2	Negative Control	31.21 ± 1.735 a***	927.5 ± 3.671 a***	2.970 ± 0.189 a***	161.0 ± 1.019 a***	56.49 a***
3	Positive Control	65.67 ± 1.104 a*** b***	818.8 ± 4.067 a*** b ^{ns}	9.830 ± 0.293 a*** b	83.81 ± 1.423 a*** b***	79.43 a* b***
4	Low dose- 250 mg/kg	44.91 ± 1.051 a*** b*** c***	866.7 ± 3.893 a*** b ^{ns} c ^{ns}	5.798 ± 0.251 a*** b*** c***	118.2 ± 0.751 a*** b*** c***	72.57 a*** b*** c***
5	High dose- 500 mg/kg	62.48 ± 0.656 a*** b*** c ^{ns}	770.7 ± 7.724 a*** b** c ^{ns}	8.906 ± 0.226 a*** b*** c ^{ns}	98.82 ± 0.265 a*** b*** c***	79.95 a* b*** c ^{ns}



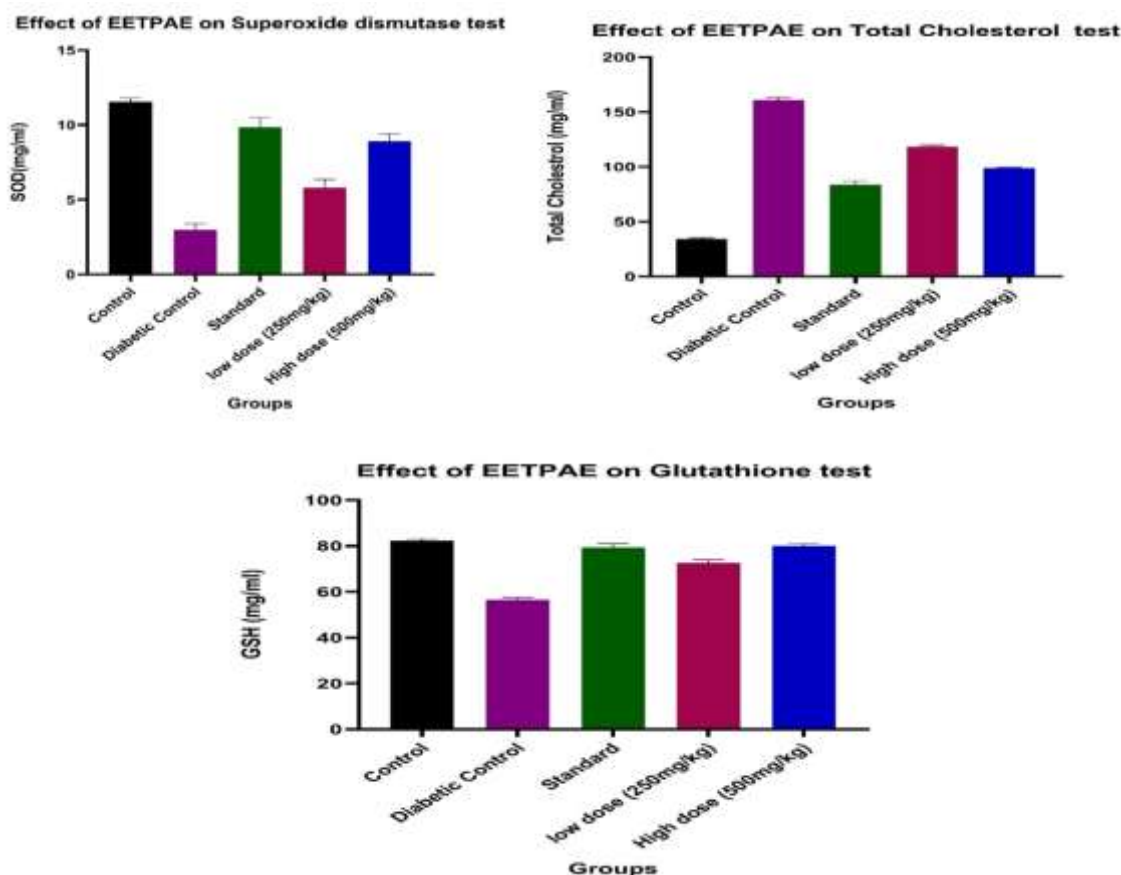


Fig: 10 Assessment of Biochemical Analysis

4.4.5 HISTOPATHOLOGY:

Group I: Control Group: Normal histological architecture. The sciatic nerve tissue showed no pathological alterations. The axons, myelin sheath, and connective tissue appeared. Represents the baseline healthy condition, serving as a standard for comparison with other groups.

Group II: Diabetic Control Group: Significant degeneration of nerve fibers, axonal swelling, and vacuolization. The sciatic nerve showed marked demyelination and inflammatory infiltration, which are characteristic of diabetic neuropathy. Indicates the damaging effect of untreated diabetes on peripheral nerves.

Group III: Standard Treated Group: Improved nerve architecture compared to Group II. Reduced degeneration and partial restoration of nerve

fibers. Treatment with the standard drug ameliorated the diabetic nerve damage to some extent, preserving nerve fiber integrity. Confirms the efficacy of standard treatment in reversing or preventing diabetic-induced nerve damage.

Group IV: Low Dose EETPAE Treated Group (250 mg/kg): Mild regeneration of nerve fibers with moderate reduction in vacuolization and axonal damage. The low dose of the test compound (EETPAE) shows a protective effect on the sciatic nerve, though less pronounced than the standard drug. Indicates potential neuroprotective action at a lower dose, but not completely restorative.

Group V: High Dose EETPAE Treated Group (500 mg/kg): Significant regeneration of nerve fibers, near-normal histology. The high dose of EETPAE demonstrated strong neuroprotective

and regenerative effects, showing better results than the low dose. Suggests a dose-dependent

effect of EETPAE in mitigating diabetic neuropathy.

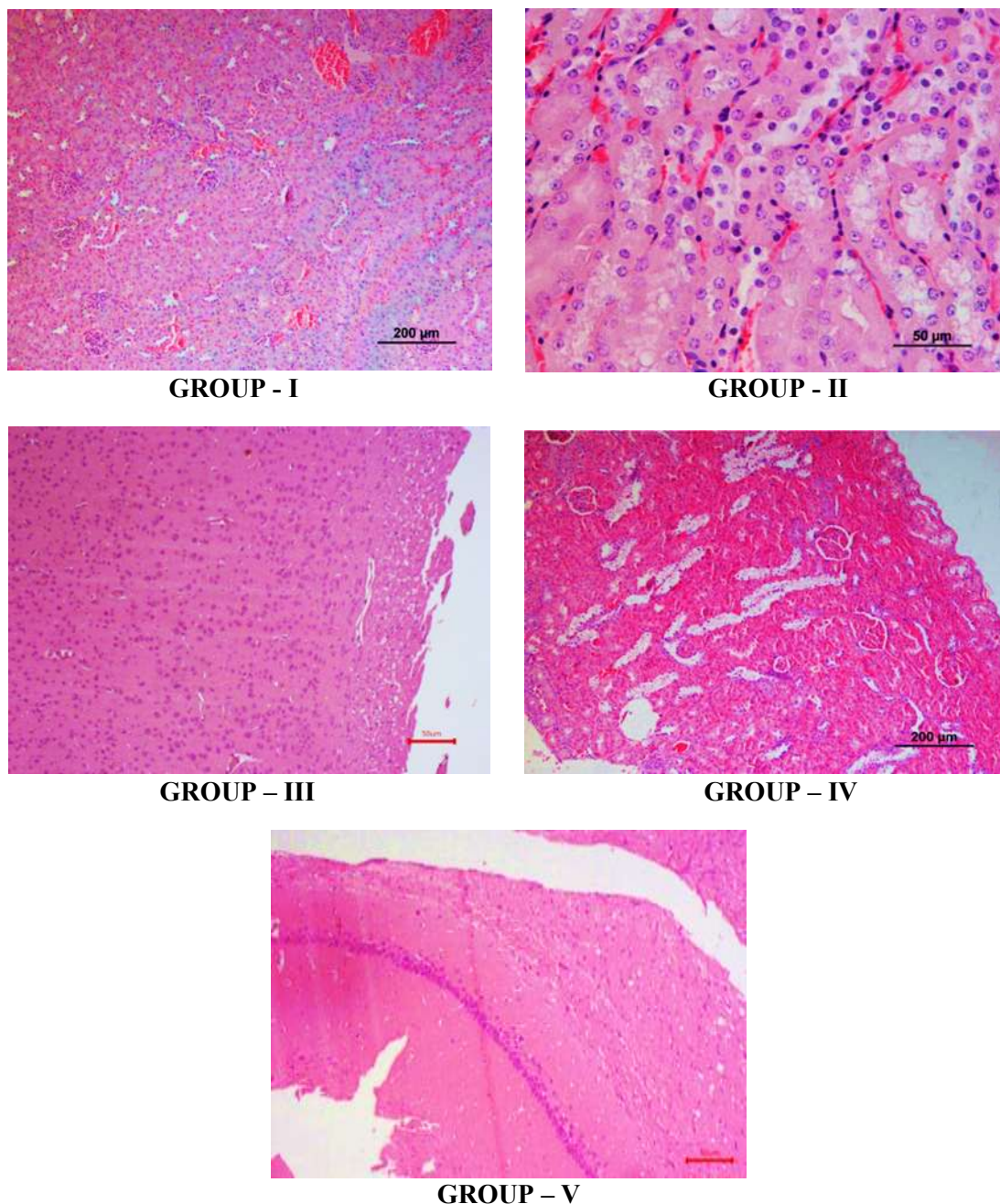


Fig: 11 Histopathology

CONCLUSION:

In conclusion, the present study demonstrates that EETPAE (Ethanollic Extract of *Tridax procumbens* & *Andrographis echiodides*) exhibits significant analgesic, antioxidant, and lipid-lowering activities in a dose-dependent manner.

The extract effectively improved pain thresholds, restored antioxidant enzyme levels (CAT, SOD, GSH), and reduced elevated free fatty acids and total cholesterol, with the high dose (500 mg/kg) showing near-comparable effects to the standard drug. These findings suggest that EETPAE holds promising potential as a natural therapeutic agent

for managing pain, oxidative stress, and metabolic imbalances, likely due to its rich phytochemical composition. Further investigations are warranted to isolate the active constituents and explore the underlying molecular mechanisms.

REFERENCES

1. "Introduction of Diabetes"- Diabetes: symptoms, causes, treatment and prevention [online], https://www.healthline.com*health.
2. "Diabetes Blue Circle Symbol". International Diabetes Federation. 17 March 2006. Archived from the original on 5 August 2007.
3. WHO | Diabetes mellitus". WHO. Retrieved 2019-03-23
4. Rockefeller, J.D. (2015). Diabetes: Symptoms, Causes, Treatment and Prevention. ISBN 978-1-5146-0305-5.
5. Saedi, E; Gheini, MR; Faiz, F; Arami, MA (15 September 2016). "Diabetes mellitus and cognitive impairments". *World Journal of Diabetes*. 7 (17): 412–22.
6. "Causes of Diabetes", National Institute of Diabetes and Digestive and Kidney Diseases. June 2014.
7. "RSSDI textbook of diabetes mellitus (Revised 2nd ed.)" Jaypee Brothers Medical Publishers. 2012. p. 235. ISBN 978-93-5025-489-9. Archived from the original on 14 October 2015.
8. "Definition of Diabetes mellitus". *Medicine Net* [online] www.medicinenet.com
9. "Diabetes- symptoms and causes"[Google],<http://www.mayoclinic.org>
10. "Complication of diabetes"- <http://www.diabetes.org.uk>
11. Bliss, Michael, 1941–2017. (2007). *The discovery of insulin (25th anniversary ed.)*. Chicago: University of Chicago Press. p. 26. ISBN 9780226058993. OCLC 74987867.
12. Von Mehring J, Minkowski O (1890). "Diabetes mellitus nach pancreas extirpation". *Arch Exp Pathol Pharmacol*. 26 (5–6): 371–387. doi:10.1007/BF01831214. S2CID 3115269
13. "Human PubMed Reference:", National Center for Biotechnology Information, U.S. National Library of Medicine.
14. Mayo Clinic Diabetes: "Prediabetes". [1]. Accessed Jan. 27, 2009.
15. "Type 1 Diabetes Mellitus". Archived from the original on 2013-07-21. Retrieved 2008-08-04
16. "The Oral Diabetes Drugs Treating Type 2 Diabetes Comparing Effectiveness, Safety, and Price" (PDF). Archived (PDF) from the original on June 15, 2013. Retrieved July 17, 2013
17. "Williams textbook of endocrinology" (12th ed.). Elsevier/Saunders. 2011. pp. 1371–1435. ISBN 978-1-4377-0324-5.
18. "Lambert P, Bingley PJ (2002)", "What is Type 1 Diabetes?". *Medicine*. 30: 1–5. doi:10.1383/medc.30.1.1.28264.
19. "Classification of Diabetes mellitus 2019". WHO. Retrieved 2020-11-09.
20. Rother KI (April 2007). "Diabetes treatment—bridging the divide". *The New England Journal of Medicine*. 356 (15): 1499–501. doi:10.1056/NEJMp078030. PMID 17429082.

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