International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1449-1465 www.ijprajournal.com ISSN: 2456-4494

Evaluation of Hydroalcoholic Extract of Tridax Procumbens L and Its Chitosan Nanoparticle against Streptozotocin-Induced Diabetic Neuropathy in Swiss Albino Mice

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Date of Submission: 18-05-2024 Date of Acceptance: 28-05-2024

ABSTRACT:
Diabetic neuropathy is one of the most prevalent complications of diabetes mellitus, affecting a significant proportion of individuals with the disease. Epidemiological studies have highlighted the substantial burden of diabetic neuropathy worldwide, with its prevalence varying depending on factors such as the duration of diabetes, glycemic control, and population characteristics.Moreover, comparison with duloxetine, a standard treatment for diabetic neuropathy, shows comparable or even superior efficacy of N-HETP, highlighting its potential as an alternative or adjunct therapy. The use of chitosan nanoparticles loaded with N-HETP further demonstrates the feasibility of nanoparticle-based drug delivery for enhancing therapeutic efficacy. The study highlights the potential of N-HETP, a novel formulation comprising chitosan nanoparticles loaded with Hydroalcoholic Extract Of Tridax Procumbens L, in alleviating streptozotocin-induced diabetic neuropathy in Wistar albino mice. Through various tests and analyses, N-HETP showed significant reductions in hyperglycemia, pain sensitivity, and improvements in motor coordination compared to untreated diabetic mice. Additionally, it enhanced antioxidant enzyme activities and preserved glutathione levels, mitigating oxidative stress and neuropathic damage associated with diabetes.

Keywords: Tridax Procumbens L, medicinal plant, Diapeticneutopathy, Nanoparticles.

I. INTRODUCTION:

Diabetes is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood glucose), or when the body cannot effectively use the insulin it produces. Raised blood glucose, a common effect of uncontrolled diabetes, may, over time, lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves. More than 400 million people live with diabetes. There are four types of diabetes mellitus, theyareType-1diabetes (previously known as insulin- dependent, juvenile or childhood-onset diabetes), Type2 diabetes (formerly called non-insulin-dependent or adult onset diabetes), Gestational diabetes (GDM) is a temporary condition that occurs in pregnancy and carries long term risk of type 2 diabetes and MODY (Maturity onset Diabetes of the young).

Diabetic neuropathy is a complication of diabetes in which nerves are damaged due to long term high levels of blood sugar or hyperglycemia. Diabetic neuropathy can affect many parts of the body including the legs, feet, bladder, heart, gastrointestinal system, and reproductive system. Diabetic neuropathy generally develops slowly over a period of months as ongoing high blood sugar levels damage the nerves of the body. Symptoms of diabetic neuropathy can includea sensation of pain, numbness, tingling, or prickling that begins in the feet. In later stages of diabetic neuropathy, the hands can be affected as well. In some cases of diabetic neuropathy, the abnormal sensations can extend to the arm, legs andtrunk.

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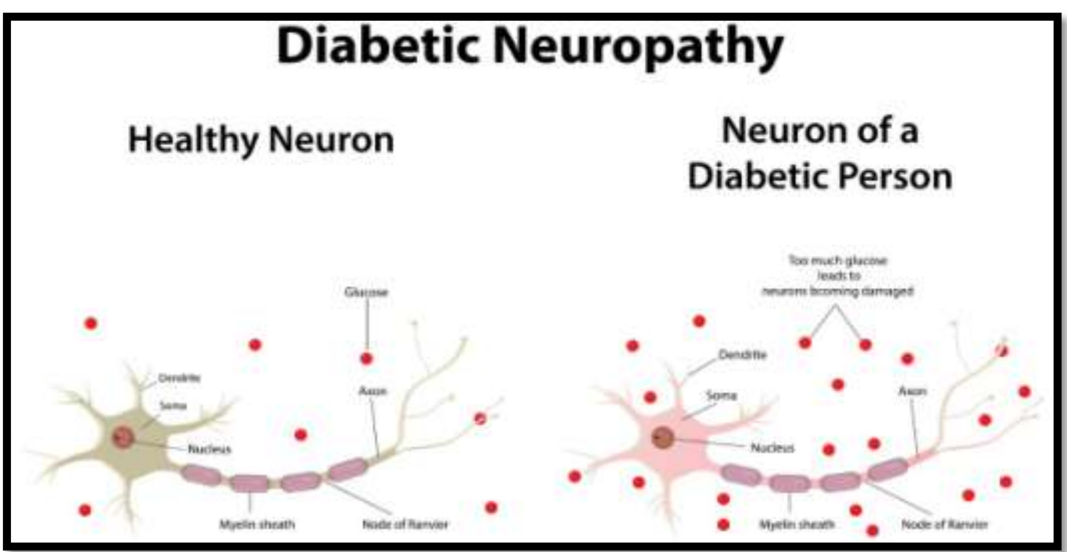

Fig. 1. Diabetic Neuropathy

II. 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. Tridaxprocumbens 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 Tridaxprocumbens's use in hair growth-promoting activity.

Fig. 2. Tridax Procumbens L

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KINGDOM : Plantae-plants
DIVISION : Magnoliphyta-flowering plant
CLASS : Magnoliopesida-dicotyledons

ORDER : Asterasles
FAMILY : Asteraceae-Aster family
GENUS : Tridax L.-tridax

SPIECES : *Tridax procumbans lin.*
ENGLISH : Coat buttons
TAMIL : Vettukaya-thalai

Fig. 3. Plant Profile Of Tridax Procumbens L

III. MATERIALS AND METHODS:

3.1 AUTHENTICATION CERTIFICATE FOR 671.15122301:

The plant material collected was identified and authenticated by by Dr. KN Sunil kumar Research officer HOD Department of pharmacognosy, Dr. P.Elankani Research officer (Siddha), Sci III-Incharge, SIDDHA CENTRAL RESEARCH INSTITUTE (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India) Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106,
Certified that the sample submitted by Maheshwaran S, M.Pharm - Final year, Aadhibhagavan College of Pharmacy, Thiruvannamalai district - 604407 was identified as:
Form No: PCOG002-ACF
Code: T15122301P
Botanical Name: **Tridax Procumbens L**
Part: Aerial Parts
Date: 15.12.2023

1.2 INDUCING AGENT (STZ):

Streptozotocin is naturally occurring chemical; used to produce Type- 1 diabetes in animal model and Type- 2 diabetes with multiple low doses. It is also used in medicine for treating metastatic cancer of islets of Langerhans.

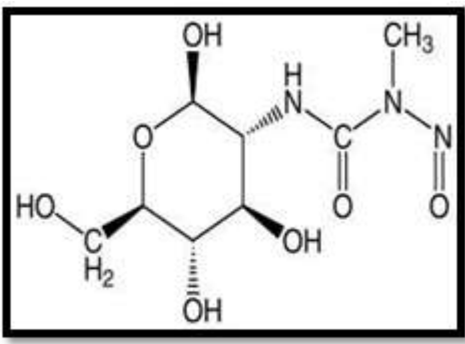

Fig. 4. 8,2-deoxy-2-{[methyl(nitroso)amino]carbonyl}amino)--D-glucopyranose (STZ)

3.3 EXTRACTION:

The whole plant ofTridaxprocumbens Lwere 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 furtheruse.A soxhlet extractor is lab equipment designed for processing certain kinds of solids. These devices allow for continuous treatment of a sample with a solvent over a period of hours or days to extract compounds. Typically, a soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

Collect dryed plant of Tridaxprocumbens L were cleaned with water & shade dried until a constant weight was obtained & subsequently

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powdered & sieved mash no 40. Powdered material 5kg was extracted with of water at 50 degree in soxhlet apparatus 1L for 72hr. dark brown semi – solid residues. 525g was obtained by evaporating the aqueous extract under reduced pressure.

Fig. 5. Hydroalcoholic Extract of Tridax Procumbens L

1.3 PHYTOCHEMICAL STUDIES:

- Determination Of Ash Values
- Total Ash
- Water Soluble Ash
- Acid In soluble Ash
- Determination Of Extractive Values
- Determination of water soluble extractive

3.4 PHYTOCHEMICAL TEST:

- Test for Carbohydrates (Molisch Test)
- Test for Alkaloids (Mayer's Test)
- Test for Steroids and Sterols (Salkowski test)
- Test for Glycosides (Legal's test)
- Test for Saponins (Foam test)
- Test for Flavonoids
- Test for Tri-terpenoids
- Test for Terpenoids (Copper acetate test)
- Tests for Tannins and Phenolic Compounds
- Test for Gums and Mucilage
- Test for Proteins and Amino acids (Biuret test, Ninhydrin test)
- Test for Fixed Oils and Fatty acids (Saponification test)

3.5 QUANTITATIV EESTIMATION OF PHYTOCONSTITUENT:

Determination Of Total Flavanoids:

Methods:

Ultra Violet spectroscopy method was carried out for the determination of total flavonoids content.

Preparation of Standard Stock solution:

Accurately weighed 25 mg of Quercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) were made with dimethyl sulfoxide.

Preparation of Test Solution:

The leaf extract was weighed accurately equal to the weight of Standard Quercetin and transferred to 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The dilution was made with dimethyl sulfoxide.

Procedure:

From the prepared solution of standard and test solutions 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10 minute at ambient temperature. After 10 minute, Standard and sample solution measure the absorbance of spectrophotometrically at 430 nm with the standard and test sample solutions.

3.6 FORMULATION AND EVALUATION OF NANOPARTICLES:

3.6.1 NP Formulation and Chitosan-Conjugation:

Extract loaded PLGA NPs was prepared by an solvent evaporation technique, a solution of Extract and PLGA in Chloroform was poured into an aqueous surfactant solution (1% w/v) and the resulting mixture was stir with magnetic stirrer to obtain a primary O/W emulsion. The O/W emulsion appears was immediately added drop-wise to an aqueous surfactant solution (1% w/v). This dispersion was further stir overnight with a magnetic stirrer at 1500 rpm. The organic solvent (chloroform) was removed under reduced pressure. Then prepare the Chitosan – PLGA coated NP was obtained by incubating a certain volume of the PLGA NP suspension with equivalent volume of 2mg/ml Chitosan solution (0.5%). This dispersion was again stir with a magnetic stirrer at 1500 rpm for 3-4 hrs at room temperature and finally the nanoparticles should be formed out.

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3.6.2 Composition Of Drug – Polymer Concentration:

S.NO	FORMULATION	DRUG CONC (mg)	POLYMER CONC		SURFACTANT
			PLGA (mg)	CHITOSAN (mg)	PVA (%)
1.	HETP-NP	50	15	9	1

Table. 1. Composition Of Extract – Polymer Ratio

3.6.3 Evaluation:
- Particle Size Distribution
- Surface Morphological Analysis
- Zeta Potential

3.7ACUTE TOXICITY STUDIES:

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines. Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4h with free access to water. The Hydroalcoholic Extract of TridaxProcumbens L (HETP) was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for first 24 hrs and after 72 hrs.

If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose wasrepeatedagaintoconfirmthetoxiceffect.Ifnomortalitywasobserved then higher (50, 300, 2000 mg/kg) doses of the plant extracts were employed for further toxicity studies.

3.8EXPERIMENTAL DESIGN:

3.8.1 Induction Of Type-I Diabetes Mellitus In Mice:

A total thirty-six (36) Mice were divided into two sets, set-1 & set-2 respectively .Set-1 consist of 6 animals and set -2 consist of 30 animals. Set-1 & Set-2 animals were subjected to an overnight fast and Only set-2 animals were injected intraperitoneally (I.P) with freshly prepared Streptozotocin (STZ), dissolved in citrate buffer at pH 4.5, at a dose of 45 mg/kg/day for five consecutive days to induce type 1 diabetes mellitus. Control mice (Set-1 animals) received equivalent volumes of citrate buffer intraperitoneally for five days while being allowed normal food intake and provided with 10% sucrose water, which was subsequently replaced with regular water after five days. Hyperglycemia was confirmed one week post-STZ injection by measuring fasting blood glucose levels using an Accu Check glucometer with blood samples obtained from the tail vein. Mice with fasting blood glucose levels exceeding 250 mg/dl were considered diabetic and included in the study.

3.8.2 Study 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 Group VI, each comprising 6 animals (n=6).

- Group-I (Control group) (n=6) receives normal saline (0.9% Nacl)
- Group-II (Negative Control group) (n=6) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + receives normal saline for 20 days after confirming diabetic neuropathy.
- Group-III (Standard group) (n=6) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Duloxetine (30 mg/kg/day) for 20 days after confirming diabetic neuropathy.
- Group-IV (Low dose group) (n=6) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Low dose (200 mg/kg/day) hydroalcoholic extract of tridaxprocumbens. (HETP) for 20 days after confirming diabetic neuropathy.
- Group-V (High dose group) (n=6) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + Low dose (400 mg/kg/day) Hydroalcoholic Extract OfTridaxProcumbens. (HETP) for 20 days after confirming diabetic neuropathy..

- Group-VI (Nano particle group) (n=6) receives Streptozotocin (STZ) 45 mg/kg/day for five consecutive days + chitosan nanoparticle loaded with Hydroalcoholic Extract OfTridaxProcumbens. (N-HETP) (50 mg/kg/day) for 20 days after confirming diabetic neuropathy.

3.9. PHARMACOLOGICAL STUDIES:

- Hot Plate Test
- Cold Plate Test
- Von Frey Filament Test
- Formalin Test
- Rota Rod Test
- Superoxide Dismutase (SOD) Activity
- Catalase (CAT)
- Glutathione Reductase (GSH)
- Glutathione Peroxidase (GPx) Activity

IV. RESULTS & DISCUSSION:

4.1 Extraction:

Drug	Aerial Part of Tridaxprocumbens L
Solvent	Hydroalcoholic 90%v/v
Colour	Dark yellowish green
Consistency	Semi solid
Percentage yield	17.5 % w/w

Table. 2. Appearance and Percentage Yield OfHETP

4.2 Preliminary Phytochemical Screening:

Results of the Preliminary Phytochemical Constituents present in Hydro alcoholic extract of Tridax procumbens L

S. No.	Constituents	Tridax Procumbens L Hydroalcoholic Extract
1.	Alkaloids	+
2.	Carbohydrates	_
3.	Protein	_
4.	Terpinoids	+
5.	Phenols	+
6.	Tannins	+
7.	Flavonoids	+
9.	Glycosides	+
10.	Saponins	_

+ = Present - = Absent

Table. 3.Preliminary Phytochemical Screening

4.3 Preliminary Phytochemical Analysis:

S.No.	Physio-Chemical Constant	Tridax Procumbens L	Limits (%W/W)
1	**Total Ash**	8.3±1.8	Not more than12
2	**Acid Insoluble Ash**	1.3±1.5	Not more than 2.5
3	**WaterSolubleExtractive**	28.3±1.6	Not less than 25
4	**Loss On Drying**	8.6	Not more than 15

Table. 4. Preliminary Phytochemical Analysis

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4.4 Quantitative Estimation:

S.No.	Concentration of standard Solution (µg/ml)	Absorbance (435nm)
1.	20	0.131
2.	40	0.169
3.	60	0.211
4.	80	0.322
5.	100	0.388
6.	TRIDAX PROCUMBENS L	0.181

Table.5. Spectrophotometric Absorbance Of Standard and Sample

From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of flavonoid present in 1gm of the extract was calculated by applying the dilution factor. The concentration of each extract obtained.

S.No.	Sample	Concentration Obtained(mg/gm)	PercentageofFlavonoids Present
1.	TRIDAX PROCUMBENS L	41.40	4.1

Table. 6. PercentageYieldOf TotalFlavonoid

4.5 Nanoparticles Evaluation:

Table. 7. Particle Size &Zeta potential value Report

S.No.	Formulation	Particle Size	Zeta potential value (mV)
1.	HETP-NP	421	-13.4

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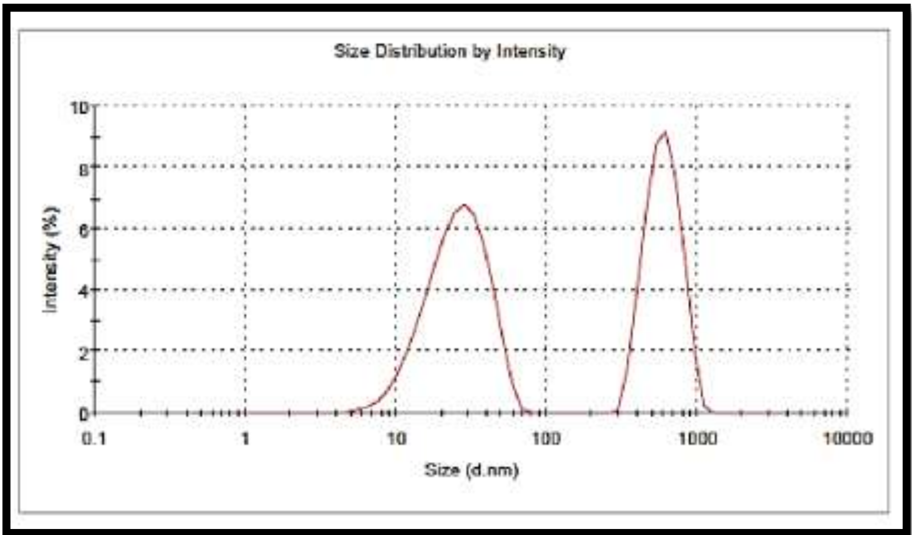

Fig. 6. Particle Size Of HETP-NP

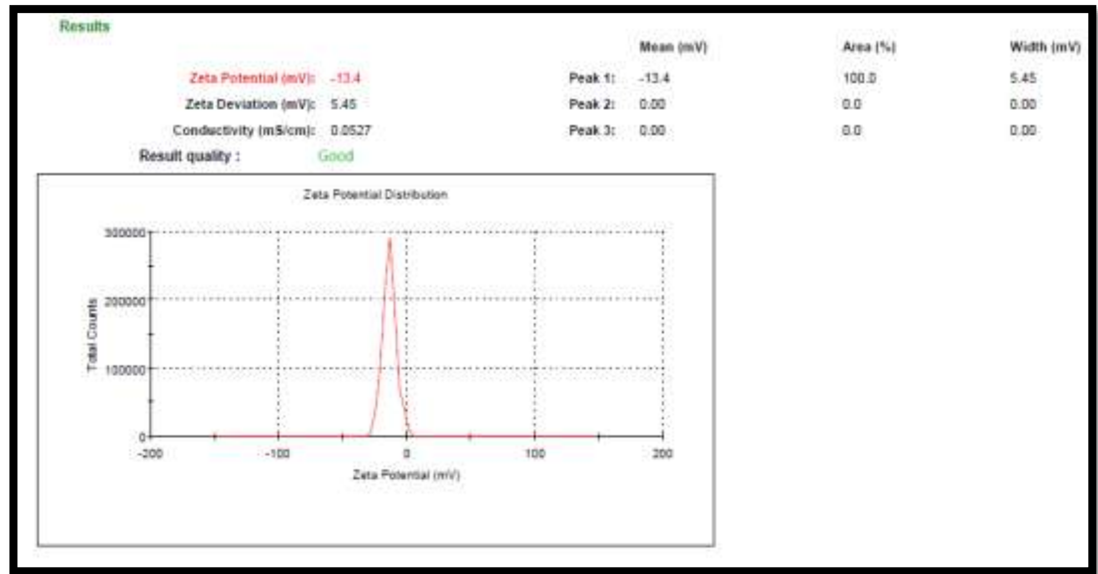

Fig. 7. Zeta Potential Of HETP-NP

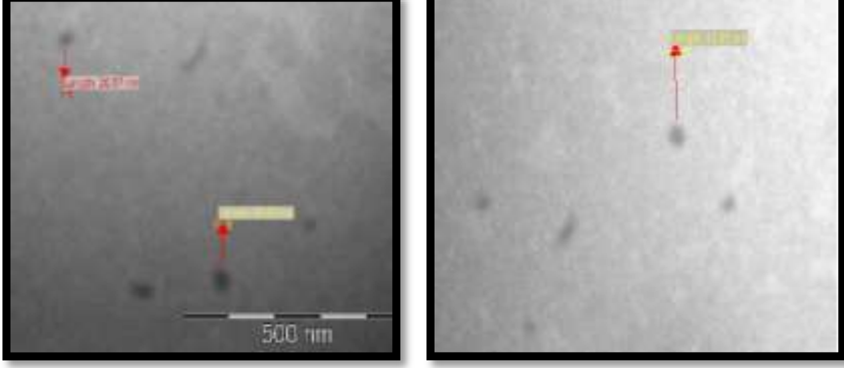

Fig. 8. Surface Morphological Analysis Of HETP-NP

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4.6 PHARMACOLOGICAL STUDY:

4.6.1 Assessement of Blood Glucose level:

S.NO.	Groups	Blood Glucose Level (MG/DL)
1	Control	97.17 ± 1.44
2	Negative Control	450.8 ± 6.76a****
3	Positive Control	422.0 ± 4.93a**** b*** c^{ns}
4	HETP- 200 mg/kg (Low Dose)	419.2 ± 2.76a**** b*** c^{ns}
5	HETP- 400 mg/kg (High Dose)	431.2 ± 4a**** b*c^{ns}
6	N- HETP – 50 mg/kg (Nanoparticle loaded with drug)	425.5 ± 3.64a**** b**c^{ns}

Table. 8. Effect of N- HETP on Blood glucose Level

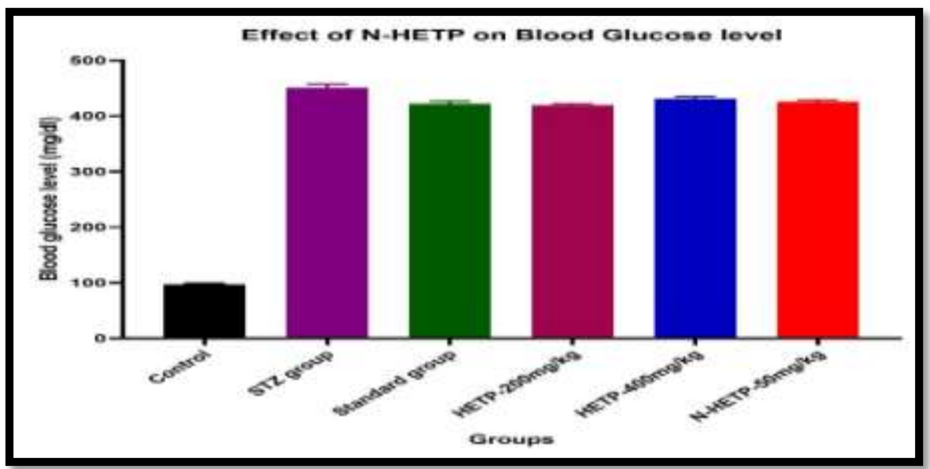

Fig. 9. Effect of N- HETP on Blood glucose Level

4.6.2 Assessement of Thermal Hyperalgesia:

S.No.	Groups	Withdrawl Latency(IN SEC.)
1	Control	16.17 ±0.4773
2	Negative Control	8.167 ± 0.3073a****
3	Positive Control	16.17 ± 1.078a^{ns} b** c^{ns}
4	HETP- 200 mg/kg	10.50 ± 0.4282a***b^{ns} c*
5	HETP- 400 mg/kg	13 ± 0.2582a** b**c^{ns}
6	N- HETP – 50 mg/kg	19 ± 1.065a^{ns} b**c^{ns}

Table. 9. Effect of N- HETP on Hot Plate Test

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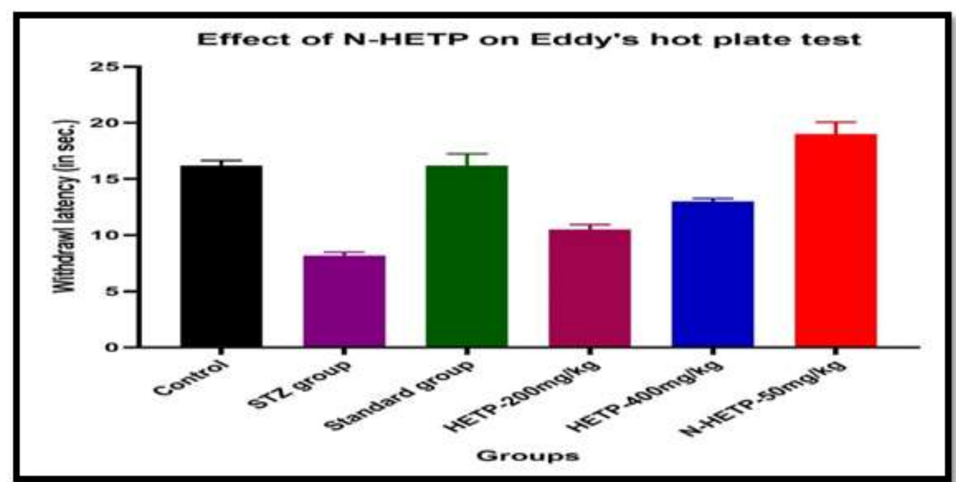

Fig. 10. Effect of N- HETP on Hot Plate Test

4.6.3 Assessement of Thermal Allodynia:

S.No.	Groups	Escape Latency (IN SEC.)
1	Control	14.17 ± 0.30
2	Negative Control	3.33 ± 0.27a****
3	Positive Control	10.83 ± 0.60a**** b****c^{ns}
4	HETP- 200 mg/kg	8.16 ± 0.19a**** b**** c***
5	HETP- 400 mg/kg	10.50 ± 0.34a**** b****c^{ns}
6	N- HETP – 50 mg/kg	14 ± 0.25a^{ns} b**** c****

Table. 10. Effect of N- HETP on Cold Plate Test

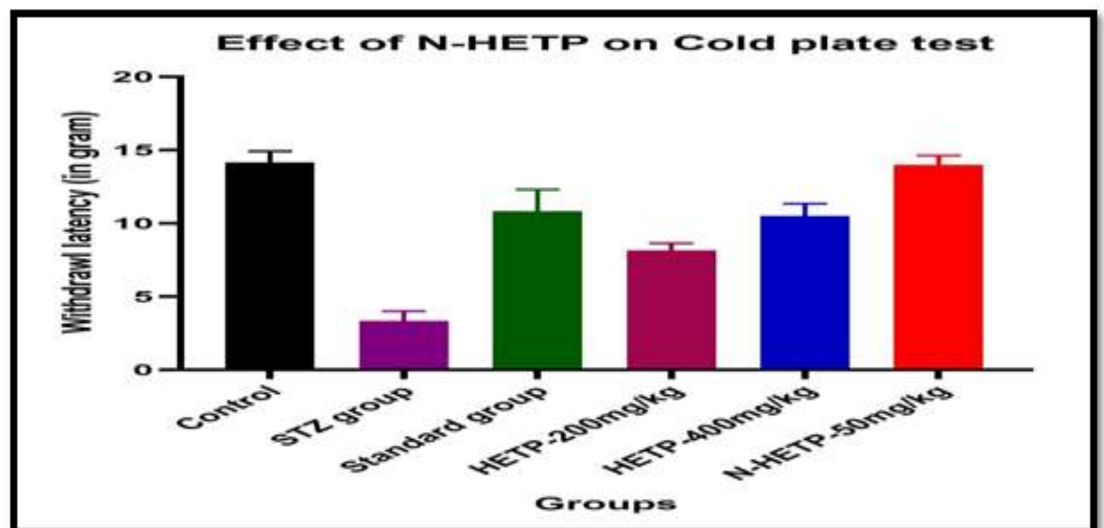

Fig. 11. Effect of N- HETP on Cold Plate Test

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4.6.4 Assessement of Mechanical Hyperalgesia:

S.No.	Groups	Escape Latency (IN SEC.)
1	Control	286.5 ± 3.640
2	Negative Control	93.33 ± 4.410a****
3	Positive Control	256.7 ± 5.869a^{ns} b***c^{ns}
4	HETP- 200 mg/kg	138.3 ± 3.333a*** b* c****
5	HETP- 400 mg/kg	216.7 ± 7.491a* b****c^{ns}
6	N- HETP – 50 mg/kg	284.2 ± 3.745a^{ns} b****c^{ns}

Table. 11. Effect of N- HETP on Von Frey Filament Test

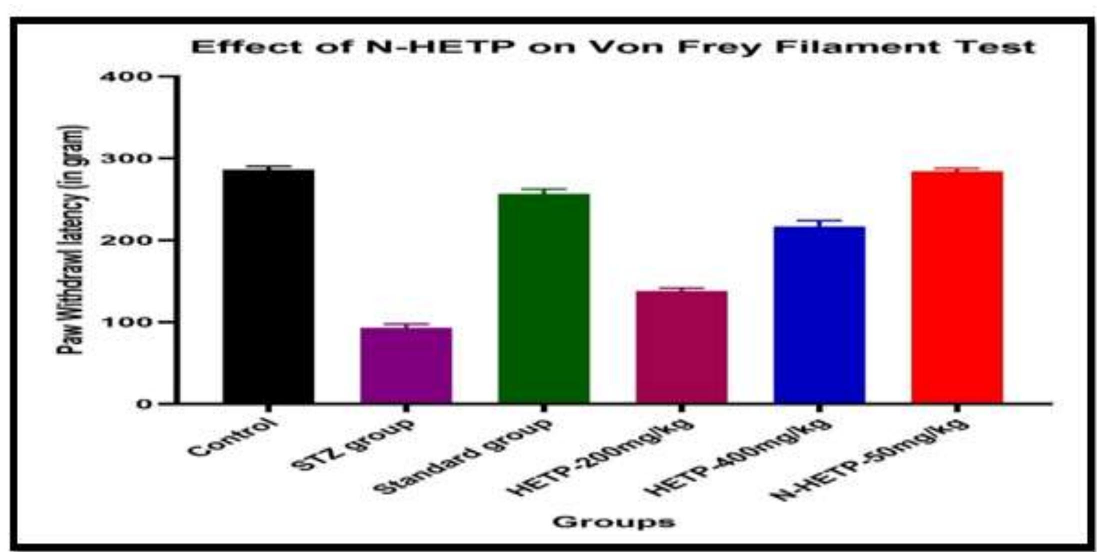

Fig. 12. Effect of N- HETP on Von Frey Filament Test

4.6.5 Effect Of N- HETP On Formalin Test:

S.No.	Groups	Formalin Test- I (Acute Phase)	
		Paw Licking (IN SEC.)	Paw Elevation (IN SEC.)
1	Control	82.17 ± 0.7032	150.3 ± 2.824
2	Negative Control	166.2 ± 3.772a****	239.7 ± 4.73a****
3	Positive Control	100.3 ± 1.406a****b****	134.0 ± 2.96a**b****
4	HETP- 200 mg/kg	134.5 ± 1.765a****b**** c****	208.7 ± 2.23a**** b****c$^{n\ ****}$
5	HETP- 400 mg/kg	110.8 ± 1.662a**** b**** c*	156.3 ± 1.64a^{ns} b**** c****
6	N- HETP – 50 mg/kg	98.50 ± 0.9916a**** b****c^{ns}	125.8 ± 2.72a**** b****c^{ns}

Table. 12. Effect of N- HETP on Formalin Test

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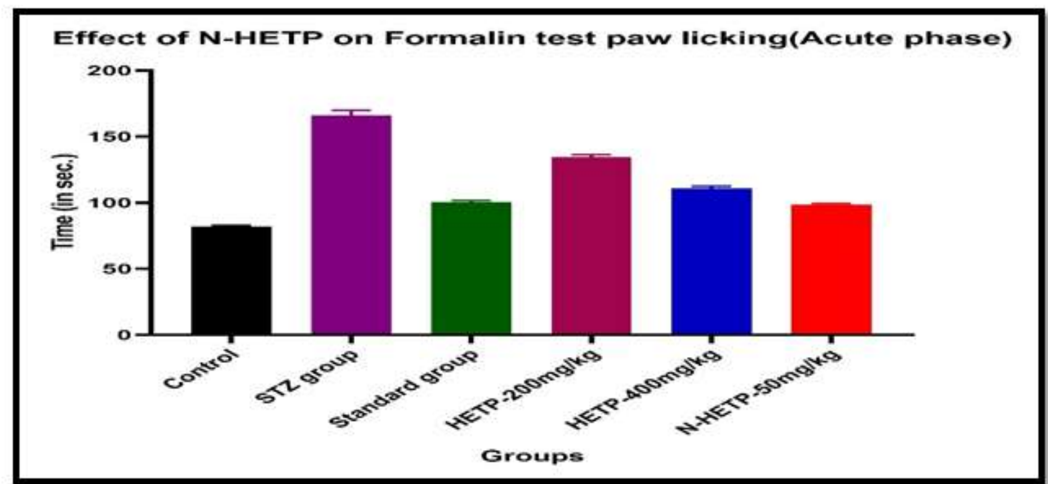

Fig. 13. Effect of N- HETP on Formalin Test Paw Licking (Acute Phase)

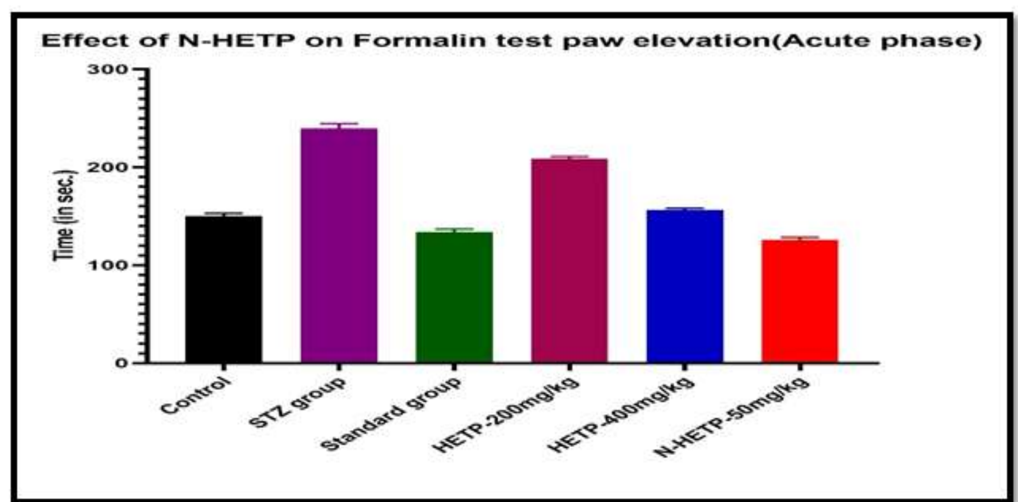

Fig. 14. Effect of N- HETP on Formalin Test Paw Elevation (Acute Phase)

4.6.6 Effect of N- HETP On Formalin Test-II (Delayed Phase):

S.NO.	GROUPS	Formalin Test-II (Delayed Phase)	
		Paw Licking (IN SEC.)	Paw Elevation (IN SEC.)
1	Control	75.17 ±1.579	144.0 ± 2.309
2	Negative Control	148.8 ± 2.242a****	302.2 ± 2.088a****
3	Positive Control	94.17 ± 1.400a****b****	127.6 ± 2.067a***b****
4	HETP- 200 mg/kg	133.7 ± 1.476a****b****c****	224.5 ± 1.839a****b****c****
5	HETP- 400 mg/kg	117.2 ± 2.242a****b****c****	161.5 ± 2.952a***b****c****
6	N- HETP – 50 mg/kg	87.17 ± 1.662a****b****c^ns	109.8 ± 3.260a****b****c***

Table. 13. Effect of N- HETP on Formalin Test-II (Delayed Phase)

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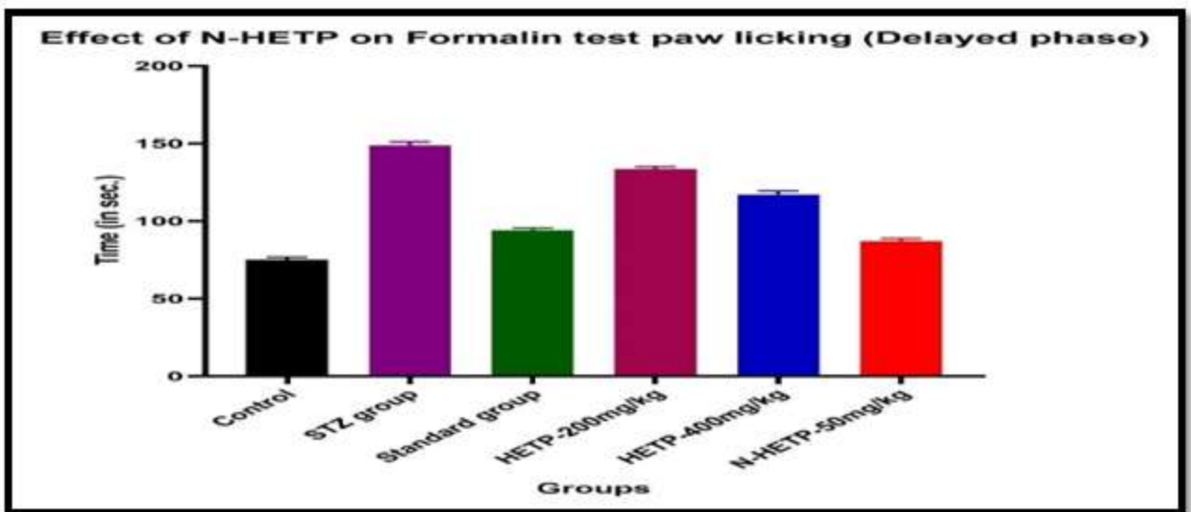

Fig. 15. Effect of N- HETP on Formalin Test Paw Licking (Delayed Phase)

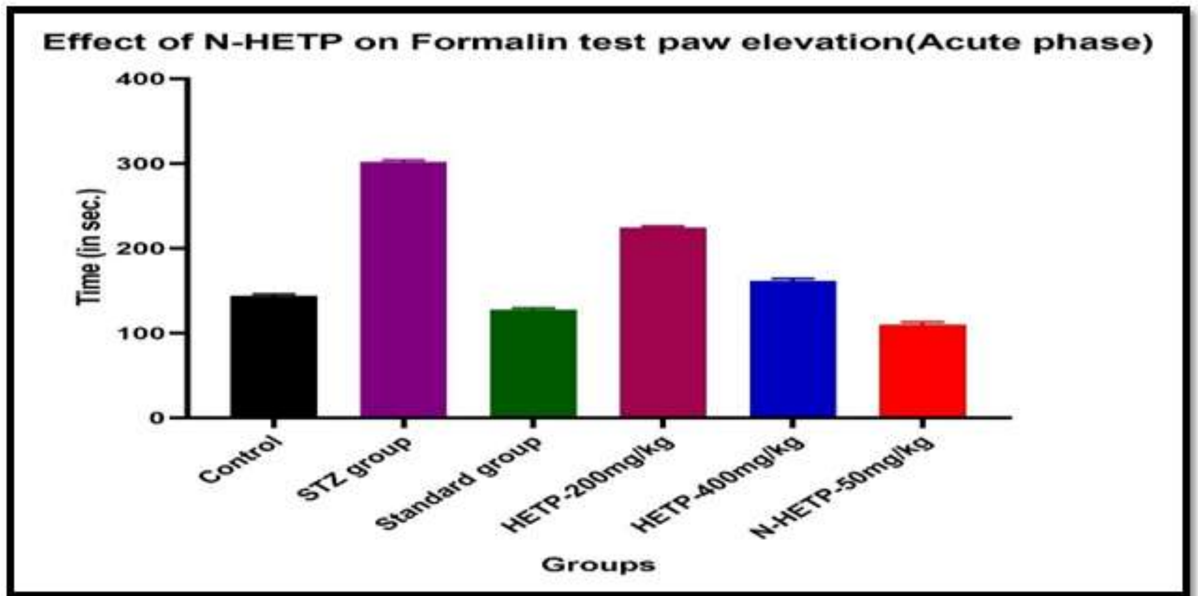

Fig. 16. Effect of N- HETP on Formalin Test Paw Elevation (Acute Phase)

4.6.7 AssessementOf Muscle Grip Strength:

S.No.	Groups	Fall Of Time(IN SEC.)
1	Control	106.5 ± 2.566
2	Negative Control	$40.33 \pm 1.745a^{****}$
3	Positive Control	$99.17 \pm 1.014a^{ns}b^{****}$
4	HETP- 200 mg/kg	$82.83 \pm 1.195a^{****}b^{****}c^{****}$
5	HETP- 400 mg/kg	$97.00 \pm 1.390a^{**}b^{****}c^{ns}$
6	N- HETP – 50 mg/kg	$121.8 \pm 1.956a^{****}b^{****}c^{****}$

Table. 14. Effect of N- HETP On Rota Rod Test

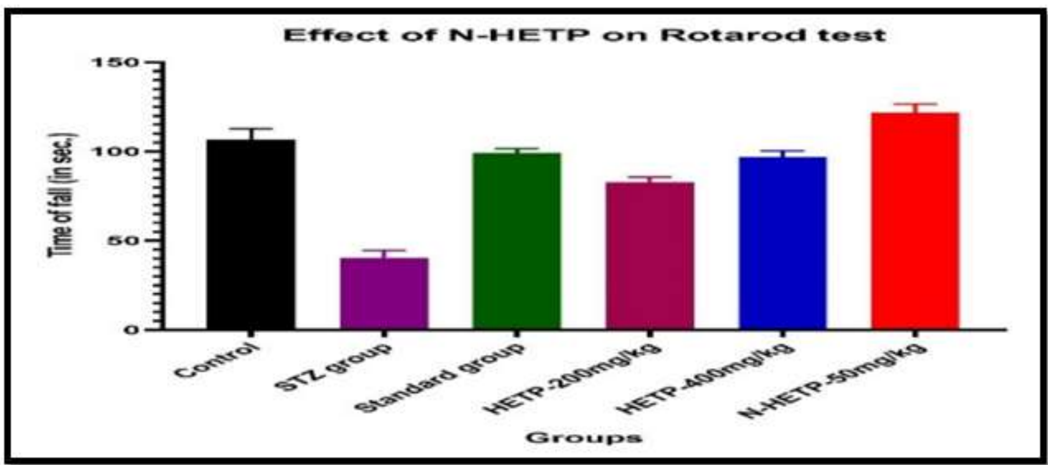

Fig. 17. Effect of N- HETP on Rotarod Test

4.6.8 Assessement Of Biochemical Analysis:

S.No.	Groups	SOD (UNITS / mg PROTEIN)
1	Control	53.00 ± 1.155
2	Negative Control	$19.00 \pm 0.5774a^{****}$
3	Positive Control	$37.83 \pm 0.7032a^{****}b^{****}$
4	HETP- 200 mg/kg	$42.67 \pm 0.7149a^{****}b^{****}c^{ns}$
5	HETP- 400 mg/kg	$51.17 \pm 0.8724a^{ns}b^{****}c^{****}$
6	N- HETP – 50 mg/kg	$64.67 \pm 2.044a^{****}b^{****}c^{****}$

Table. 15. Effect of N- HETP On Superoxide Dimutase

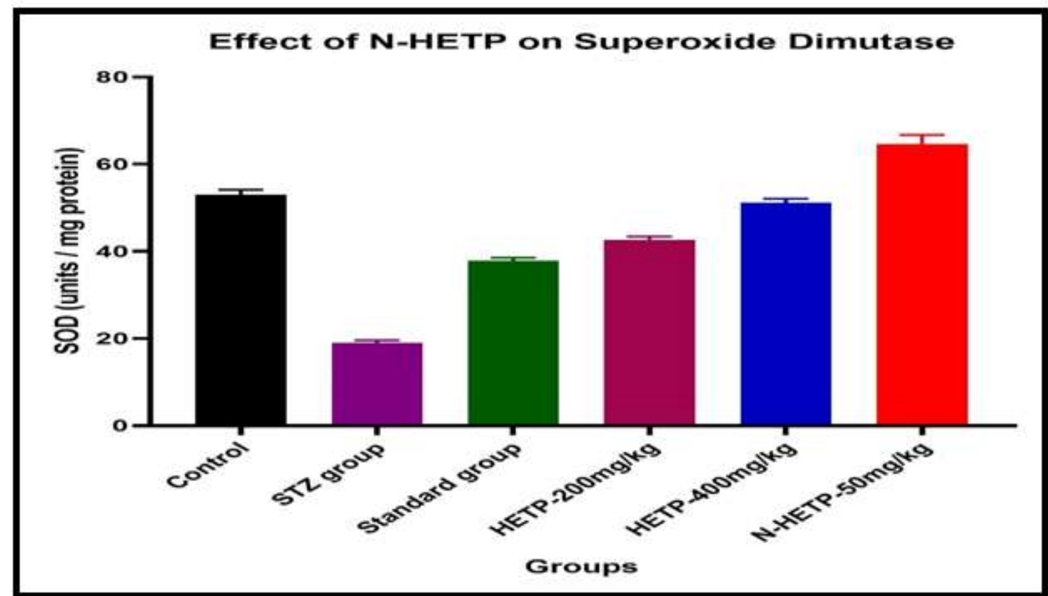

Fig. 18. Effect of N- HETP on Superoxide Dimutase

4.6.9 Effect of N- HETP on Catalase:

S.NO	GROUPS	CAT (UNITS / mg PROTEIN)
1	Control	63.67 ± 0.5578
2	Negative Control	35.33 ± 1.054a****
3	Positive Control	52.83 ± 1.078a****b****
4	HETP- 200 mg/kg	44.33 ± 0.7149a****b*** c***
5	HETP- 400 mg/kg	54.50 ± 1.455a***b****c^{ns}
6	N- HETP – 50 mg/kg	68.83 ± 1.922a^{ns}b**** c****

Table. 16. Effect of N- HETP On Catalase

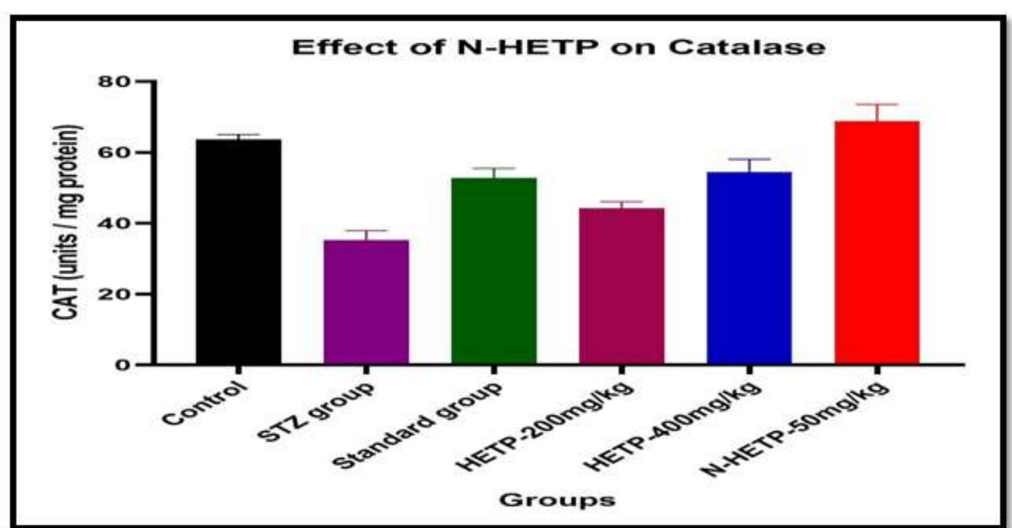

Fig. 19. Effect of N- HETP onCatalase

4.6.10 Effect of N- HETP on Glutathione Peroxidase:

S.NO	GROUPS	GPX (UNITS / mg PROTEIN)
1	Control	164.8 ± 4.152
2	Negative Control	84.00 ± 3.521a****
3	Positive Control	150.6 ± 1.806a*b****
4	HETP- 200 mg/kg	104.6 ± 1.806a****b*** c****
5	HETP- 400 mg/kg	136.2 ± 1.241a****b**** c*
6	N- HETP – 50 mg/kg	153.0 ± 1.871a^{ns}b****c^{ns}

Table. 17. Effect of N- HETP OnGlutathione Peroxidase

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 1449-1465 www.ijprajournal.com ISSN: 2456-4494

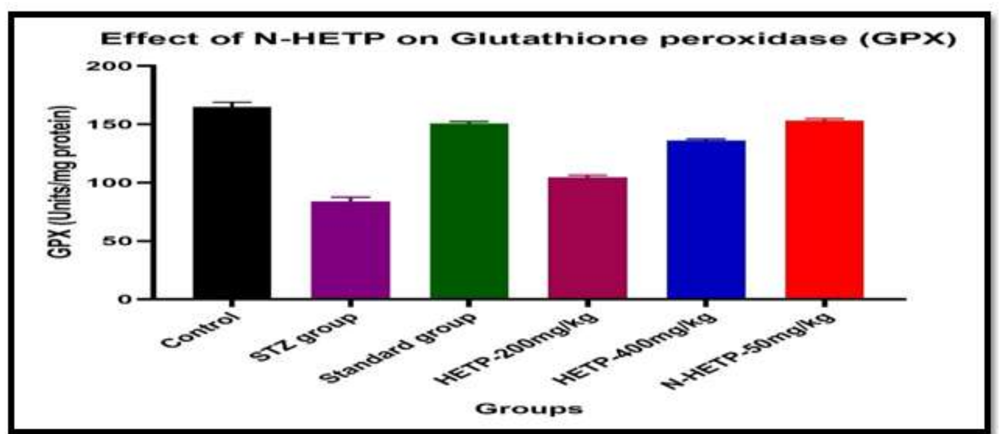

Fig. 20. Effect of N- HETP onGlutathione Peroxidase

4.6.11 Effect of N- HETP on Glutathione:

S.NO	GROUPS	GSH (UNITS / mg PROTEIN)
1	Control	43.17 ± 1.424
2	Negative Control	14.67 ± 0.4216a****
3	Positive Control	33.50 ± 0.9916a****b****
4	HETP- 200 mg/kg	22.67 ± 0.7149a****b**** c****
5	HETP- 400 mg/kg	39.33 ± 0.9189a^{ns}b**** c**
6	N- HETP – 50 mg/kg	50.33 ± 1.358a***b**** c****

Table. 18. Effect of N- HETP On Glutathione

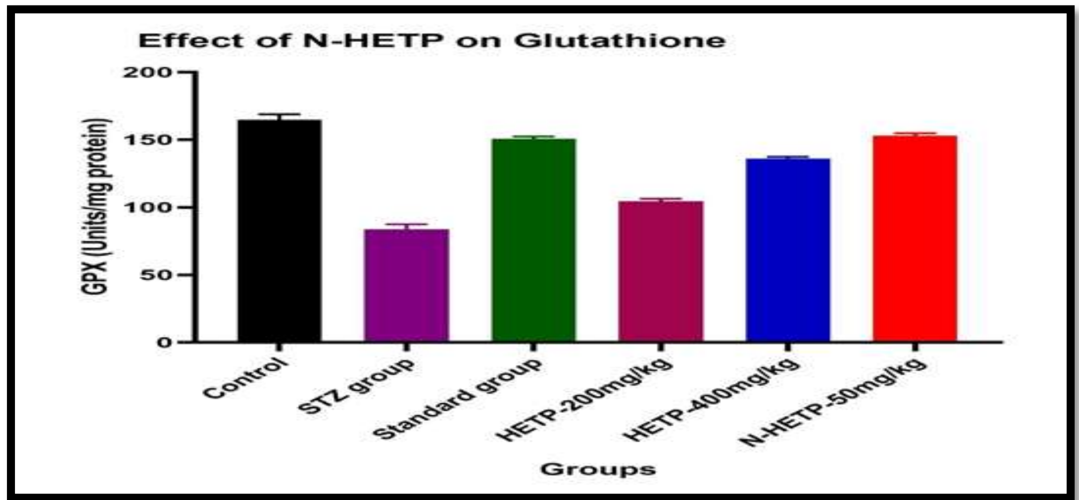

Fig. 21. Effect of N- HETP onGlutathione

V. CONCLUSION:

In conclusion, the study highlights the potential of N-HETP, a novel formulation comprising chitosan nanoparticles loaded with Hydroalcoholic Extract Of Tridax Procumbens L, in alleviating streptozotocin-induced diabetic neuropathy in Wistar albino mice. Through various tests and analyses, N-HETP showed significant reductions in hyperglycemia, pain sensitivity, and improvements in motor coordination compared to untreated diabetic mice. Additionally, it enhanced antioxidant enzyme activities and preserved glutathione levels, mitigating oxidative stress and neuropathic damage associated with diabetes. The results suggest N-HETP as a promising therapeutic agent, offering targeted delivery of bioactive compounds to manage diabetic neuropathy effectively. Further research is needed to understand its mechanisms and optimize dosing for clinical applications, potentially improving the quality of life for diabetic patients.

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