Research Article

Evaluation of Diabetic Neuroprotective Effect by *Ipomoea sepiaria* on Acrylamide Induced Neuropathic Pain in Rats

G. Shanthini Nachiar*[1], S. Kokila,[2] Dr.S. Amudha[3], T. Sheela,[4] K. Vaithiyanathan[5]
School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai-119, Tamil Nadu, India.
C.L. Baid Metha College of Pharmacy, Chennai-97, Tamil Nadu, India.
Mariyam College of Pharmacy, Tirunelveli, Tamil Nadu, India.
***Corresponding author's E-mail:** shanthini31011980@gmail.com

Received: 23-12-2023; **Revised:** 04-02-2024; **Accepted:** 10-02-2024; **Published on:** 15-02-2024.

ABSTRACT

Neuropathic pain associated with peripheral nerve injury is characterized by sensory abnormalities such as unpleasant and abnormal sensations (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia) According to ethnomedical claim leaf decoction of *Ipomoea sepiaria* is used for nervous disorders. Hence the Ethanolic extract of *Ipomoea sepiaria* has been evaluated for acrylamide-induced painful neuropathy in rats. Wistar rats (either sex, 150-200gm) were employed in the present study. Animals were maintained at laboratory diet and allow to free access to water ad libitum. They were housed in the animal house and exposed to normal cycle of light and dark. The Acrylamide 30mg/kg was administered by intraperitonially once in three days, for 24 consecutive days to induce painful neuropathy. All the groups of animals were subjected to sensory behavioral tests such as paw cold allodynia, hot plate test, and tail immersion test in order to assess the degree of nociceptive threshold on certain day intervals, ie.0,3, 6,9, 12,15, 19,21 & 24thday.Six groups, each comprising six Wistar rats, were employed in the study. Rats were not subjected to administration of vehicle and acrylamide and were kept for 24 days. Behavioural tests were employed to assess nociceptive threshold on different day's such as 0,3, 6,9, 12.15,19,21 & 24th. All the animals were sacrificed according to CPCSEA guidelines at the end of the 24th day. Sciatic nerve of the animals was isolated and homogenated to estimate biochemical markers such as TBARS, reduced glutathione, total protein and total calcium levels. Histopathological studies also carried out with the distal portion of the sciatic nerve.

Keywords: Acrylamide, Pregabalin, Ethanolic leaf extract of *Ipomoea sepiaria*, Wistar rats, TBARS, GSH.

INTRODUCTION

Neuropathy is a collection of disorders that occurs when nerves of the peripheral nervous system (the part of the nervous system outside of the brain and spinal cord) are damaged. The condition is generally referred to as peripheral neuropathy, and it is most commonly due to damage to nerve axons. Neuropathy usually causes pain and numbness in the hands and feet. It can result from traumatic injuries, infections, metabolic disorders, and exposure to toxins.

Neuropathy can affect nerves that control muscle movement (motor nerves) and those that detect sensations such as coldness or pain (sensory nerves). In some cases - autonomic neuropathy - it can affect internal organs, such as the heart, blood vessels, bladder, or intestines. Pain from peripheral neuropathy is often described as a tingling or burning sensation. There is no specific length of time that the pain exists, but symptoms often improve with time - especially if the neuropathy has an underlying condition that can be cured. The condition is often associated with a number of diseases, and pressure or trauma, but many cases have no known reason and other wise called as idiopathic neuropathy.

Neurophathies may also be categorized based on a functional classification (motor, sensory, autonomic, or mixed) or the type of onset (acute - hours or days, subacute - weeks or months, or chronic - months or years). The most common form of neuropathy is (symmetrical) peripheral polyneuropathy, which mainly affects the feet and legs on both sides of the bod**y.**

About 30% of neuropathy cases are considered idiopathic, which means they are of unknown cause. Another 30% of neuropathies are due to diabetes. In fact, about 50% of people with diabetes develop some type of neuropathy. The remaining cases of neuropathy, called acquired neuropathies, have several possible causes, including Trauma or pressure on nerves, Nutritional problems and vitamin deficiencies, often from a lack of B vitamins. Alcoholism, often through poor dietary habits and vitamin deficiencies. Autoimmune diseases, such as lupus, rheumatoid arthritis, and Guillain-Barre syndrome. Motor nerve damage usually leads to symptoms that affect muscles such as muscle weakness, cramps, and spasms. It is not uncommon for this type of neuropathy to lead to a loss of balance and coordination. Patients may find it difficult to walk or Peripheral neuropathic pain has been frequently observed in patients with cancer, AIDS, longstanding diabetes, lumbar disc syndrome, herpes infection, traumatic spinal cord injury, multiple sclerosis and stroke. Moreover, post thoracotomy, post-herniorrhaphy, post-mastectomy, and post-sternotomy have also been associated with neuropathic pain.

MATERIALS AND METHODS

Plant Material

Ethanolic leaf extract of ***Ipomoea sepiaria,*** **[EEIS]**

Animals

Wistar rats (either sex, 150-200gm) were employed in the present study. Animals were maintained at laboratory diet and allow to free access to water ad libitum. They were housed in the animal house and exposed to normal cycle of light and dark. The experimental protocol was duly approved by the institutional animal ethical committee and animals were maintained as per CPCSEA guideline. (Committee for the purpose of control and supervision of experiments on Animals Ref no:14024/E1/4/2011) of the Dean, Madurai medical college, Madurai.

Chemicals

Acrylamide was purchased from CDH, chemicals New Delhi. All other chemicals used for this study were of analytical grade.

Induction of Neuropathic pain

The Acrylamide 30mg/kg was administered by intraperitonially once in three days, for 24 consecutive days to induce painful neuropathy. All the groups of animals were subjected to sensory behavioral tests such as paw cold allodynia, hot plate test, and tail immersion test in order to assess the degree of nociceptive threshold on certain day intravels, ie.0,3, 6,9, 12,15, 19,21 & 24thday.[1-20]

Experimental Design

Six groups, each comprising six Wistar rats, were employed in the present study.

Group I (Normal control group)

Rats were not subjected to administration of vehicle and acrylamide and were kept for 24 days. Behavioural tests were employed to assess nociceptive threshold on different day's i.e. 0,3, 6,9, 12.15,19,21 & 24th. All the animals were sacrificed according to CPCSEA guidelines at the end of the 24th day. Sciatic nerve of the animals was isolated and homogenated to estimate biochemical markers such as TBARS, reduced glutathione, total protein and total calcium levels. Histopathological studies also carried out with the distal portion of the sciatic nerve.[21-35]

Group II (Acrylamide 30mg/kg, i.p)

Acrylamide (30mg/kg, i.p) was administered to normal rats once in three days for 24 consecutive days. Behavioural tests and Biochemical parameters were assessed as described in group I.

Group III (Ethanolic extract of EEIS 100mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 100mg/kg, p.o was administered two hours before each acrylamide injection (acrylmide was administered once in three days) for 24 consecutive days. Behavioural tests and Biochemical parameters were assessed as mentioned in group I.

Group I V (Ethanolic extract of EEIS 200mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 200mg/kg,p.o, was administered two hours before each acrylamide injection (Acrylamide was administered once in three days) for 24 consecutive days. Behavioral tests and Biochemical parameters were assessed as mentioned in group I.

Group V (Ethanolic extract of EEIS 500mg/kg treated group)

Ethanolic leaf extract of *Ipomoea sepiaria* 5 mg/kg,p.o was administered two hours before each acrylamide injection (Acrylmide was administered once in three days) for 24 consecutive days. Behavioural tests and Biochemical parameters were assessed as mentioned in group I.

Group VI (Pregabalin 10mg/kg, treated group)

Pregabalin (10mg/kg, p.o) was administered two hours before each acrylamide injection (acrylamide was administered once in three days) for 24 consecutive days. Behavioral tests and Biochemical parameters were assessed as mentioned in group I.

SENSORY BEHAVIOURAL ASSESMENT

Paw cold allodynia

Cold allodynia of the hind paw was assessed using acetone drop method as described by Choi et al (1994) with slight modification, Assessing the reactivity to non-noxious cold chemical stimuli. The rats were placed on the top of a wire mesh grid allowing access to the hind paw. Acetone(0.1ml) was sprayed on the plantar surface of hind paw of rat and time taken for the withdrawal of the hind paw from the mesh surface was noted ,with cut-off time of 60 seconds.[36-40]

Hot plate test

Heat thermal sensitivity of the hind paw was assessed by using Eddy's hot plate as described method of Eddy et at, with slight modification for assessing the degree of noxious thermal sensation. The rats were placed on the top of a preheated (52 ± 0.5ºc) hot plate surface, allowing access to the hind paw withdrawal response to degree of the nociceptive threshold. The cut- off time of 20 seconds was maintained.

Tail immersion test

Tail immersion test was carried out to assess the spinal heat thermal sensitivity. Tip of the rat's tail was immersed in heat noxious temperature (52 ± 0.05ºc) till the tail was withdrawn. Thermal heat hyperalgesia was assessed by duration of the tail withdrawal reflex. The cut off time of 10 s was maintained.

Biochemical estimation of markers of oxidative stress

All the groups of animals were sacrificed after 24th days by cervical dislocation and the sciatic nerve was isolated immediately and used for the biochemical estimation. The distal most part of the nerve, which was used for histopathological study. Freshly excised sciatic nerve homogenate (10%) was prepared with 0.1M Tris HCl buffer (PH-7.4) and the homogenate was kept in ice water for 30 min and centrifuged at 4ºc (2000g, 10 min). The supernatant of homogenate was separated and which was used to estimate following biochemical markers.

Estimation of total protein content

The protein concentration was estimated according to the method of Lowry et al[5] using bovine serum albumin as a standard. The absorbance was determined spectrophotometrically at 750nm.

Estimation of total calcium

Total calcium level was estimated in sciatic nerve as described method of Severtnghaus and Ferrebee and Muthuraman. Total calcium level was estimated in sciatic nerve. The sciatic nerve homogenate was mixed with 1ml of trichloroacetic acid (4%) as in ice cold condition and centrifuged at 1500g for 10 mins. The clear supernatant was used for the estimation by atomic emission spectroscopy at 556 nm.

Estimation of reduced glutathione

Equal quantity of sciatic nerve homogenate was mixed with 10% trichloroacetic acid and the mixture was centrifuged to separate proteins. To 0.01ml of this supernatant, 2ml of phosphate buffer (PH-8.4), 0.5ml of 5, 5' dithiobis (2-nitrobenzoic acid) and 0.4ml of distilled water were added. Mixture was vortexed and the absorbance was taken at 415nm within 15mins. The concentration of reduced glutathione was expressed as µg/mg of protein.

Estimation of TBARS

The thiobarbituric acid reactive substances (TBARS) level was estimated as per the spectrophotometric method described by Ohkawa et al method, to each test tube, 0.5 ml of supernatant,0.5 ml normal saline,1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of thiobarbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added. The test tubes were kept for boiling at 95º C for one hour. To each test tube, 3 ml of n-butanol was added and mixed well. These test tubes were centrifuged at 3000rpm for 10 minutes. The separated butanol layer was collected and read in a spectrophotometer against blank at 535 nm. Concentration of thiobarbituric reactive substances was expressed in terms of nmol of malondialdehyde per mg of protein.

Table 1: Effects of Ethanolic extract of *Ipomoea sepiaria* on tissue biochemical changes

Groups	TBARS (nmol/mg of protein)	GSH (µg/mg of protein)	Total calcium (ppm/mg of protein)
Normal	13.20 ± 0.23	69.37 ± 2.53	1.93 ± 0.79
Acrylamide	56.41 ± 0.70 [a]	12.64 ± 3.15 [a]	16.87 ± 1.31 [a]
EEIS (100 mg/kg)	29.2 ± 0.82 [b]	31.78 ± 2.96 [b]	7.16 ± 1.04 [b]
EEIS (200 mg/kg)	21.2 ± 0.85 [b]	54.63 ± 3.94 [b]	5.48 ± 1.17 [b]
EEIS-C (5 mg/kg)	20.55 ± 0.33 [b]	59.51 ± 2.76 [b]	3.64 ± 0.95 [b]
Pregabalin	15.13 ± 0.51 [b]	61.64 ± 3.36 [b]	2.72 ± 0.86 [b]

Data were expressed as mean ± SD (n=6).; EEIS, (Ethanolic extract of *Ipomoea sepiaria*); EEIS-C, (Ethanolic extract of *Ipomoea sepiaria* isolated compound); [a]*P < 0.05* Vs. normal group.; [b]*P < 0.05* Vs. acrylamide control group.; Digits in parentheses indicate dose in mg/kg.; TBARS-Thiobarbituric acid reactive substance; GSH-Reduced glutathione; *Ipomoea sepiaria*.

Histopathological examination

Samples of sciatic nerve were fixed with 10% formalin and cut in to sections with 4µm thickness, staining was done by using haematoxylin and eosin. Nerve sections were analysed qualitatively under light microscope (45x) for axonal degeneration and fibres dearrangements.Effect of *Ipomoea sepiaria* on acrylamide induced histopathological changes were shown in following figures.

Fig 1-5 shows cross section of sciatic nerve of normal acrylamide, EEIS 100 mg, 200 mg and 5 mg/kg.p.o, Treated groups respectively. Fig-1 showed normal histopathology of sciatic nerve, In fig-2 shows acrylamide induced axonal degeneration and dearrangement of nerve fibres. Fig-3,4 and 5 pretreatment of *Ipomoea sepiaria* showed decrease in the acrylamide induced histopathological changes of sciatic nerve.

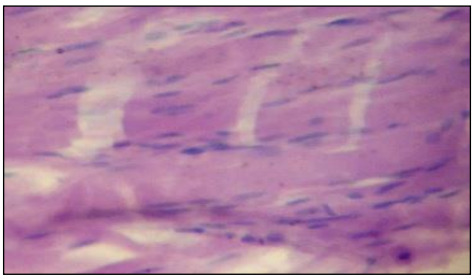

Figure 1: Normal Control Group-I

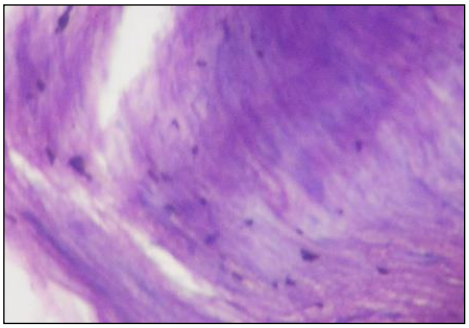

Figure 2: Acrylamide Treated Group-II

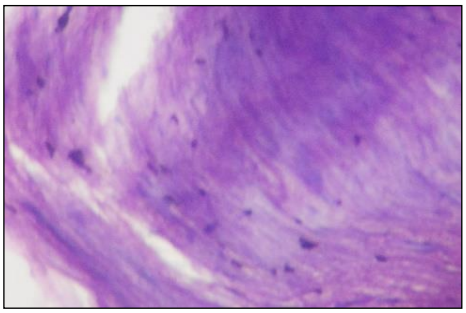

Figure 3: EEIS treatment 100 mg/kg Group-III

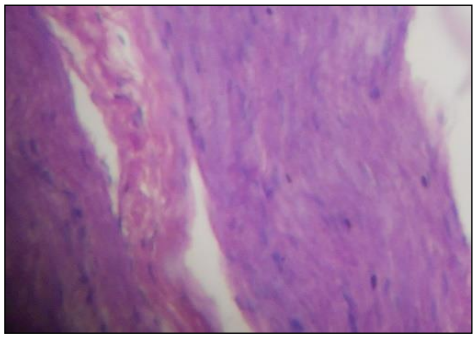

Figure 4: EEIS treatment 200 mg/kg Group-IV

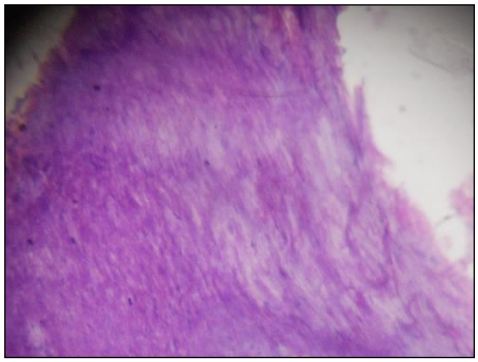

Figure 5: EEIS treatment 500 mg/kg Group-V

Statistical analysis

All the results were expressed as standard error of mean (SEM). Data obtained from behavioural tests were statistically analysed by using two-way repeated ANOVA, while data of biochemical parameters were analysed using one way ANOVA. In both cases, Tukey's multiple range tests were applied for post-hoc analysis. A value of $p<0.05$ was considered to be statistically significant.

Neuroprotective activity of flavonoids against toxin induced neuropathy was attributed due to their antioxidant potential.

RESULTS AND DISCUSSION

Effect of EEIS on Paw cold allodynia

Acrylamide induced toxicity of Sciatic nerve resulted significant development of non-noxious cold chemical allodynia, noted by decrease in hind paw withdrawal threshold after 6th day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for cold allodynia was improved by the administration of EEIS (100,200&500mg). Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with EEIS (100,200 & 500mg/kg,p.o.

Effect of EEIS on paw heat hyperalgesic test

Acrylamide induced toxicity of Sciatic nerve resulted significant development of noxious thermal hyperalgesia noted by decrease in hind paw withdrawal threshold after 6th day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by the administration of EEIS (100,200&500mg/kg, p.o).Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with EEIS (100,200&500mg/kg, p.o, $P < 0.05$).

Tail immersion test

Acrylamide induced toxicity of Sciatic nerve resulted significant development of noxious thermal hyperalgesia noted by decrease in tail withdrawal threshold after 6th day of acrylamide intoxication as compared to normal control group. Acrylamide induced, decrease in nociceptive threshold for thermal hyperalgesia was improved by the administration of EEIS (100, 200 & 500mg/kg, p.o).Treatment of pregabalin also produced similar effects. However statistically significant attenuation was recorded in all the groups treated with both EEIS (100,200 & 500mg/kg, p.o).

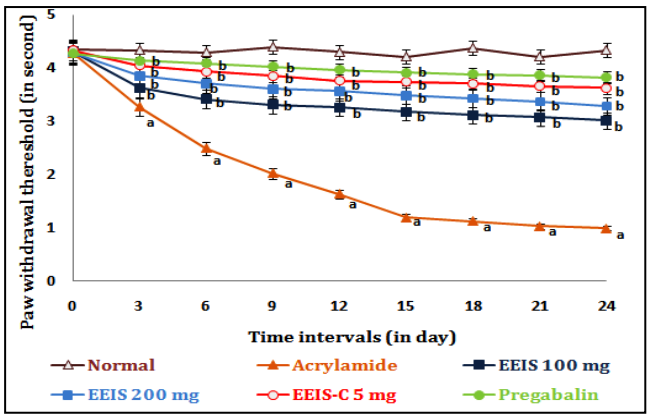

Figure 6: Effect of Ethanolic extract of *Ipomoea sepiaria* by paw cold allodynia

Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of *Ipomoea sepiaria*); EEIS-C,(Ethanolic extract of *Ipomoea sepiaria* isolated compound)

[a]*P < 0.05* Vs. normal group.

[b]*P < 0.05* Vs. acrylamide control group.

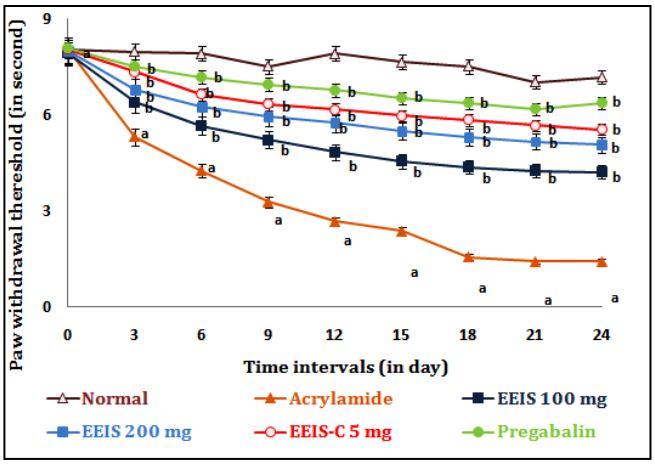

Figure 7: Effect of Ethanolic extract of *Ipomoea sepiaria* on hot plate test

Data were expressed as mean ± SD (n=6).

EEIS, (Ethanolic extract of *Ipomoea sepiaria*); EEIS-C,(Ethanolic extract of *Ipomoea sepiaria* isolated compound);

[a]*P < 0.05* Vs. normal group.

[b]*P < 0.05* Vs. acrylamide control group.

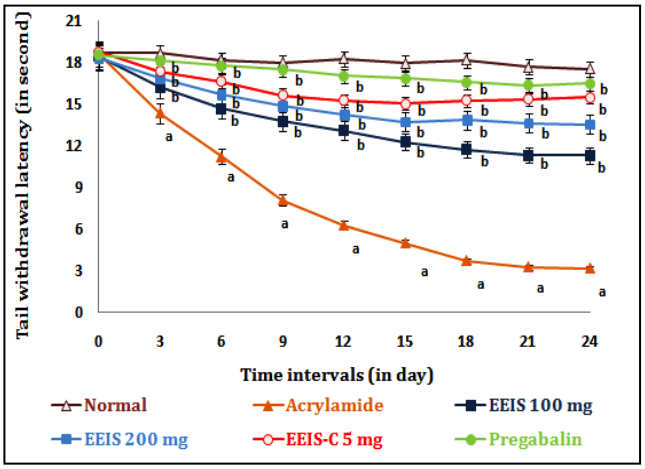

Figure 8: Effect of Ethanolic extract of *Ipomoea sepiaria* on tail immersion test

Data were expressed as mean ± SD (n=6).

EEIS,(Ethanolic extract of *Ipomoea sepiaria*); EEIS-C,(Ethanolic extract of *Ipomoea sepiaria* isolated compound)

[a]*P < 0.05* Vs. normal group.

[b]*P < 0.05* Vs. acrylamide control group.

Effect of EEIS on oxidative stress markers

Acrlyamide induced sciatic nerve intoxication resulted in significant rise in TBARS, (56.41nmol, p<0.05) total calcium level (16.87ppm, p<0.05) and decrease in the level of reduced glutathione (12.64 µg/mg, p<0.05) after the 24th day of drug administration as compared to normal control group. Administration of EEIS and isolated mixture compound attenuated acrylamide induced rise in sciatic nerve tissue level of TBARS and total calcium, and the decreased level of reduced glutathione. Administration of EEIS (100 mg/kg., p.o) resulted attenuation of TBARS (29.2 nmol, p<0.05) and total calcium (7.16 ppm, p<0.05) and rise in the level of reduced glutathione (31.78µg/mg,p<0.05). EEIS treatment (200mg/kg., p.o) resulted the reduction of TBARS (21.2 nmol, p<0.05) and total calcium (5.48 ppm, p<0.05) and increased in the level of reduced glutathione (54.63 sµg/mg,p<0.05). Administration of EEIP (500 mg/kg.p.o) was found to be decreased TBARS (20.55 nmol, p<0.05) and total calcium (3.64 ppm, p<0.05) and elevated the level of reduced glutathione (59.51µg/mg,p<0.05).

Effect of histopathological changes

Acrylamide induced toxicity of sciatic nerve resulted in significant histopathological changes assessed in cross section of the sciatic nerve. In the cross section, acrylamide induced axonal degeneration and dearrangement of nerve fibres were observed. Administration of EEIS (100mg,200mg & 500 mg/kg, p.o) significantly attenuated acrylamide induced axonal degeneration and dearrangement of nerve fibres.

CONCLUSION

In the present study, EEIS and isolated mixture compound-II treatment normalize acrylamide induced sciatic nerve intoxication mediated behavioural (heat hyperalgesia [paw & tail], cold allodynia) and biochemical abnormalities (TBARS, total calcium & reduced glutathione). Acrylamide toxicity causes the “dying back” neuropathy in rats (distal axonopathy). Acrylamide is a neurotoxic agent, is used in industries which involved in the manufacture of dye and fibre, polymer production, gel electrophoresis and water treatment. Acrylamide exposure leads to central-peripheral distal axonopathy in peripheral nervous system.

The underlying mechanism for the cause of acrylamide intoxicated neuropathy is still not yet understood. Recently oxidative stress has been demonstrated to be one of the key mechanisms in many chemical- induced cell injuries. Oxidative stress in the cells or tissues refers to enhanced generation of ROS and/or depletion of antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants, potentially leading to damage.

If the intracellular reactive oxygen species level increases may lead to damage the mitochondria, lipid peroxidation, elevated cytokine production and cell death. ROS generation in tissues is effectively scavenged by enzymatic antioxidant system, (such as SOD, GSH-Px, CAT, and GR) and non-enzymatic antioxidants (such as GSH, Vitamin A, C, and

E). This present study revealed that TBARS level of the acrylamide treated group was found to be elevated and it was found to be decreased significantly in EEIS (100mg/kg 200mg/kg&500mg/kg; P < 0.05) and isolated mixture compound (500mg/kg; P < 0.05) treated animals.

These findings indicate that acrylamide induced lipid peroxidation denoted by elevated TBARS level was markedly controlled by EEIS treatment at 100mg,200mg & 500mg/kg, p.o. Documented studies revealed that ROS can attack the polyunsaturated fatty acid in the bio membrane to initiate the free radical chain reaction.

Acrylamide treatment significantly reduces the non-enzymatic endogenous antioxidant GSH level and the EEIS treatment 100mg,200mg & isolated mixture compound 5mg/kg, p.o significantly increases the level of GSH. The above data indicate that EEIS treatment resulted elevation of endogenous antioxidant GSH. GSH which can effectively scavenge free radicals directly and indirectly which is the major non enzymatic antioxidant in cells.

GSH plays an important role in antioxidant defense, nutrient metabolism, and regulation of cellular events. GSH deficiency contributes to oxidative stress, which takes effect in the pathogenesis of many diseases, e.g., Alzheimer's disease and neuropathy.

Documented studies showed that conjugation with glutathione (GSH) is a mechanism for the detoxification of acrylamide. Glycinamide, an active neurotoxic metabolite of acrylamide, can also conjugate with GSH. The acrylamide induced depletion of GSH may make the nerve tissue more sensitive to the oxidative stress which is significantly reversed by EEIS treatment.

Acrylamide treatment increases total calcium level and treatment of EEIS at 100mg/kg, 200mg/kg and isolated mixture compound 5mg/kg, significantly reduces the total calcium level. The noted decrease in calcium level with EEIS may be attributed to its antioxidant effect, as free radicals are well reported to increase calcium ions. However, the possible action of EEIS on decrease in calcium level may not also be ruled out. Moreover, increase in calcium ions is also associated with increase in oxidative stress. So, the noted antioxidant effects of EEIS may also be ascribed secondary to decrease in calcium ions. Pregabalin treatment also increases GSH level, decreases TBARS and total calcium level. Oxidative stress may impair axonal membrane which was attenuated by EEIS treatment. EEIS showed significant antioxidant activity in ***invitro*** assays like DPPH and H_2O_2. Neuroprotective effect of EEIS against acrylamide induced neuropathy may be due to the antioxidant potential of this extract, which has been proved by both ***in vivo*** and ***invitro*** antioxidant assays. Antioxidant property of EEIS may be due to the presence of tannins and flavonoids.

Quercetin has already been reported for its neuroprotective effect against alcohol induced neuropathy by attenuating thermal hyperalgesia and also through modulation of membrane bound inorganic phosphate enzyme and inhibition of release of oxides, Inflammation mediators such as MDA (Melodi aldehyde), MPO (Myeloperoxidase), and nitric oxide.

Quercetin has proven to protect against the development of diabetic neuropathy by inhibition of lipid peroxidation and restoration of antioxidant enzyme in diabetic rats, thus reverse the oxidative stress induced changes in nerve physiology of diabetic rats are reported earlier.

Phytochemical studies revealed that Compound mixture-II consist of two compounds and one of the identified constituent may be quercetin, Administration of compound mixture –II to the acrylamide intoxicated rats resulted attenuation of behavioural parameters such as paw and tail heat hyperalgesia (P< 0.05) and cold allodynia (P<0.05) and biochemical parameters such as TBARS (P<0.05) total calcium (P<0.05) and reduced GSH (P<0.05) as well as histopathological changes. Neuroprotective effect of compound mixture-II may be due to the presence of quercetin in this mixture. Isolated compound mixture and EEIS possess therapeutic potential on acrylamide induced biochemical and histopathological changes in rats. These ameliorative effects may be attributed due to the anti-oxidative and neuroprotective potential of EEIS and isolated compound mixture-II and quercetin may be responsible for these activities.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Pareda-Miranda R, Escalanate–Sanchez E, Escobeda-Martinez C. Characterization of lipophilic pentasaccharides from beach morning glory *Ipomoea pes-caprae*. J Natural Products 2005; 56(12) :1724-1726.
2. Singh V, Pandey M, Srivastava V. Seed mucilage from *Ipomoea dasysperma.* Fitoterapia 2007; 78(3): 268-270.
3. Kappor A, Mahor R, Vaisham Payan N, Gautam N. Antifungal spectral of some petal extracts. Geobies 1981; (2): 66-67.
4. Khare AK, Srivastava MC, Tewa JP, Puri JN, Singh S, Ansari NA. A preliminary study of anti-inflammatory activity of *Ipomoea turpethium* (NISHOTH). Indian Drugs 1982; 19(6): 224-228.
5. Mujumdar AM, Marathe H, Ghaskadbi SM. Pharmacology of *Ipomoea leari.* Indian Drugs 1983; 20(8): 307-310.
6. Da Silvo Filho AA, Correa Lima RMO, De Andrade Chiapett A, Do Nascimento SC, De Souza IA, Demello JF, Veith HJ. Anticancer and antimicrobial glycosides from *Ipomoea bahiensis.* Phytochem 1986; 25(5): 1077-1081.
7. Pongprayoon U. Pharmacologic studies on the Thai medicinal plant *Ipomoea caprae*. Acta pharmaceutica Nordica 1991; 3(3): 186.
8. Reza MS, Khan MOF, Islam MA, Chowdhury AKA. *In vitro* antimicrobial activity of *Ipomoea fistula*. Fitoterapia 1994; 65(5): 465-466

9. .Reynols WF, Yu M, Enriquez RG, Gonzaler H, Leon I, Magos G, Villareal ML. Isolation and characterization of cytotoxic and antibacterial tetrasaccharides, glycosides from *Ipomoea stans*. J Natl Products 1995; 58(11): 1730-1734.
10. Navarro-Ruiz A, Delamora GP, Villanuev Michel MI, Dominguez JR, Bastidas-Ramirez BE, Quezada-Arellano JD, Rui Madrigal B. Anticonvulsant effect of aqueous, hydro alcohol and chloroform extracts from *Ipomoea stans* root in the rat. Phytotheraphy Res 1996; 10(3): 242-244.
11. Matsui T, Kobayashi EM, Sugita F, Terahara N, Matsumoto K. Antihyperglycemic effect of discylated anthocyanin derived from *Ipomea batatas* cultivar ayamurasakri can be achieved through the alpha glucosidase inhibitory action. J Agric Food Chem 2002; 50(25): 7244-7248.
12. Barnes CC, Smalley MK, Kindscha, MKP, Loring K, Sheeley H. Characterization of a tuberculosis resin glycoside from the prairimoplant, *Ipomoea leptophylla.* J Natl Prod 2003; 66(11): 1451-1462.
13. Dhembare AJ and Sangle S. Effect of plant extracts on some important pathogenic bacteria. J Exptl Zool 2003; 6(2): 299-300.
14. Haueza IM, Fonseca ESM, Paulino CA, Haraguchi M, Gorniak SL. Evaluation of immunomodulatory activity of *Ipomoea carnea* on peritonial cells of rats. J Ethnopharmacol 2003; 87: 181- 186
15. Yoshimot M, Kurataa RK, Fuji M, Hou DX, Ikeda K, Dome TY, Osako M. Phenolic composition and radical scavenging activity of sweet potato (*Ipomoea batata)* derived schochu distillery by products treated Koji. Biosci Biotech, Biochem 2004; 68(12); 2477-2483.
16. Prasad KN, Prasad MS, Shivamurthy GR, Aradhya SM. Callus induction from *Ipomea aquatic*(Forsk) leaf and its antioxidant activity. J Biotech 2006; 5(1): 107- 111.
17. Ferreria AA, Amarlal FA, Durate IDG, Oliveria PM, Alves RB, Silveria D, Azevedo AO, Raslan DS, Castro MSA. Antinociceptive effect from *Ipomoea cairica* extract. J Ethnopharmacol 2006; 105(1-2): 148-153.
18. Dattachoudry M, Choudhury R, De B, Paul SB. Physiochemical characterization and anti-fungal, CNS depressant activity of certain ethnomedicinal plants of Tripura State, India. Biosci Biotechnol Res Asia 2007; 4(1): 145-148.
19. Sokeng SD, Rokeya B, Kannan FM, Junaida K, Zitech P, Ali L, Ngounou G, Lontsi D, Kamtchouching G. *Ipomoea aquatic* extracts on glucose absorption using a perfused rat intestional preparation..Fitoterapia 2007; 78(7-8): 526-529.
20. Hamsa TP, Kuttan G. Antioxidant activity of *Ipomoea obscura*. Amala Research Bulletin 2007; 27: 24-34.
21. Vimala Y, Elizabeth KM, Srilakshmi Selvi G. Antimicrobial activity of *Ipomoea kentrochules* on pathogenic microorganisms and microbial isolates on spoiled vegetables. Asian J Micro, Biotech & Environmental Sci 2007; (1): 41-44.
22. Rivera IL, Mirron Lopez G, Molina Salinar GM, Herrera–Ruiz M, Alonso-cortes D, Navaneze Vazquez GR, Cos MY, Fernandez S. Trianthinic acids from *Ipomoea tyriantia* and their anti-mycobacterial activity, cytotoxicity and effects on the central nervous system. J Natl Prod 2008; 71(10): 1686-1691.
23. Chimkode R, Patil MB, Jallapure SS, Agarwal N, Tripathi R, Tripathi A, Patil NR. Wound healing activity of tuberous root extracts of *Ipomoea batatas*. Adv Pharmacol & Toxicol 2009; 10(3): 69-72.
24. Dass.N., Ray.B, Mahapatra S.K and Pothal R.K. Microbiological potentiality of *Ipomoea sepiaria* Roxb (Convolvulaceae).International Journal of Research in Pharmaceutical and Biomedical sciences.2011:2(2):500-501.
25. Vijayan.N,Barreto Ida,Dessai Seema, Dhuei Shital, D'Silva Riva and Rodeigues Astrida. Antimicrobial activity of ten common herbs, commonly known as Dashapushpam from kerala, India. Asian Journal of Microbiology Research. 2010;4(2):2357-2362.
26. M.Abdul Latif, Sharmina Akter, Shahjahan Kabir, M.Ansar Ali, M.Anwar Hossain and M.Loothfor Rahman.Efficacy of some organic Amendments for the control of ufra Disease of Rice.Bangladesh journal of microbiology. 2006;23:118-120.
27. Syed Shayfur Rahman, Md.Mizamur Rahman, Shameem Ara Begum, Mohammad mizamur, Rahman Khan, Md.Mosharef Hossain Bhuiyan. Investigation of Sapindus mukorossi Extracts for Repellency, Insecticidal Activity and plant growth regulatory effect. Journal of Applied Research. 2007;3(2):95-101.
28. Arunachalam muthuram, Amteshwar singh Jaggl,Nimal singh, Dhandeep singh "Ameliorative effects of amiloride and pralidoxime in chronic constriction Injury and vincristine induced painful neuropathy in rats" European journal of Pharmacology, 2008;587:104.
29. Ross and Wilson "Anatomy and physiology in health and illness" 9th edition:188.
30. Amteshwar singh Jaggi, Nirmal singh, Vivek Jain "Animal Models Neuropathic pain" Fundamental and Clinical Pharmacology.2011 Feb;25(1): 1-28.doi: 10.1111/j.1472-8206.2009.00801.x.PMID:20030738.
31. Hanaa, H.Ahmed, Gamal A.Elmegeed, El-Sayed M.El- Sayed. Mervat M.Abd-Elhalim, Wafaa Gh. Shousha, Reham W.Shafic. Potent neuropathy role of novel melatonin derivatives for management of central neuropathy induced by acrylamide in rats. European journal medicinal chemistry, 2010;45:5452-5459.
32. Eddy N.B, Touch berry C.F, Lieberman J.E, Synthetic analgesics: I. Methadone isomers and derivatives. J. Pharmacol Exp Ther, 1950;98:121-137.
33. Chaplan S.R, Bach F.W, Posrel J.W, Chung J.M, Yaksh J.L.Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Methods. 199;53:55-63.
34. Necker R, Hellen R.F,Noxious thermal input from the rat tail, modulation by desending inhibitory influences, Pain, 1978;4:231-242.
35. Okhawa H, Ohesli N, Yasik, Assay of lipid peroxidation in animal tissue by TBARS, Analyst Biochem.1979:95:351-358.
36. Ellman G.L. Tissue sulfhydryl groups, Arch. Biochem Biophys. 1959;82:70-77.
37. Severinghaus J.W, Feru bee J.W, Calcium determination by flame photometry: methods of serum, urine and other fluids. J. Biol Chem. 1950;187:621-630.

38. Muthuraman A, DiwanV, JaggiAS, SinghN, SinghD. Ameliorative effects of *Ocimum santum* in sciatic nerve transection-induced neuropathy in rats, J .Ethanopharmacol 2008;120:56-62.

39. Sudoh T, Desai S.P, Haderer A.E, Sudoh S, Gerner P, Anthony D.L, De Girolami U, Wang G.K. Neurologic and histopathologic evaluation after high volume intrathecal amitriptyline. Anaesth. Pain Med, 2004;29:434-440

40. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ Protein measurement with Folin phenol reagent. J Biol Chem. 1951;193:265-275.

41. Honda K, Casaderus G, Peterson R.B, Perry G, Smith M.A,Oxidative stress and redox active iron in Alzheimer's disease Ann Ny Acad Sci. 2004;1012:179-182.

42. Glass J.D, Culver D.G, Levery A.I, Nash N.R.Very early activation of m-calphin in pheriperal nerve during wallerian degeneration. 2002.

43. Carrido S.G, Sensi S.L, Yin H.Z, Weiss J.H Ampa exposures induced mitochondrial Ca (2+) overload and ROS generation in spinal motor neurons in vitro. J. NeuroSci. 2000;20:240-250.

44. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods.1983;65:55-63.

45. Monks, A., et al., Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. Journal of the National Cancer Institute. 1991;83:757-766.

46. Mahesh Chand Meena, Vidya Patni. Isolation and identification of Flavonoid "Quercetin" from *Citrullus colocynthis*. Asian J.Exp.Sci., 2008;22:137-142.

47. Fatemeh Fathiazad, Abbas Delazar, Royal Amiri. Extraction of flavonoids and quantification of Rutin from waste Tobacco Leaves. Iranian journal of pharmaceutical research. 2006;3:222-227.

48. Michele Morsch, Leury G.J.Girardi, Valdir Cechinel-Filho, Christiane Meyre-Silva and Clovis Antonio Rodrigues. Separation of flavonoids *Aleurites moluccana* Leaves using Chitosan Modified with Heptaldehye.NIQFAR. 2004;59C:649-652.

49. KumikoIshige, David Schubert, Yutaka Sagara Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radical Biology & Medicine, 2001;30(4):433-446.

50. Anjaneyulu M, Chopra K, Quercetin, a bioflavonoids attenuates thermal hyperalgesia in a mouse model of diabetic neuropathic pain, prog Neuropsycho pharmacol Biol Psychiatry 2003;27(6) :1001-1005.

51. Davis W.Lamson Matthew S, Antioxidants and cancer III; Quercetin Altern Med Rev, 2000;5(3):196-208.