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Putative Role of Ethanolic Extract of *Vernonia cinerea* in the Amelioration of Chemotherapy Induced Neuropathic Pain in Mice

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ABSTRACT

Cancer chemotherapy is associated with a plethora of morbidities among which neuropathic pain is another one. The pathology underpinning chemotherapy induced neuropathic pain can be multifarious, however, dearth of effective medication largely plagues the quality of life of such patients. A good rationale can be found behind narrowing down on herbal alternatives namely methanolic extracts of *Vernonia cinerea* for which anti-cancer potential has already been reported. Hence we have carried out a pilot study for evaluating the protective potential of the methanolic extract of the plant against paclitaxel induced neuropathic pain in mice. Our evaluation has been based on standard paradigms focusing on neuromotor, oxidative and histopathological assessments and TNF α Assessment. We have found significant improvisation in groups treated with both pregabalin and extract, the amelioration being largely graded in nature. Hence our research has opened up the doors of a newer horizon of herbal alternatives available for chemotherapy induced neuropathic pain, however further look out into the domain is avidly awaited for.

Keywords: NP: Neuropathic Pain, CC: Cancer Chemotherapy, HA: Herbal Alternatives

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INTRODUCTION

As we know neuropathic pain is an age old menace in developed and developing countries. According to International Association for Study of Pain (IASP) neuropathic pain is defined as pain initiated or caused by injury or dysfunction of the somatosensory pathway. The injury or dysfunction may involve peripheral or central nervous system structure characterized by pain, numbness, tingling in extremities and slow nerve conduction .[1] Epidemiologically neuropathic pain has a greater prevalence in the global prospective .So we decided to zero in on neuropathic pain in our work ahead. As suggested in the literature the course of the neuropathic pain is often complicated due to the multifactorial etiology under pinning the condition which demands closer overview. The pivotal contours may be featured as pain signaling changes, ion channel alteration, second order nociceptive neuronal alteration, environmental factors . In the modern treatment strategy having some limitation such as Gabapentin associated with sedation whereas Pregabaline associated with dizziness, blurred vision. However in the modern treatment strategy chemotherapy induced peripheral neuropathic pain may occur in cancer patient either early in the course of chemotherapy or after repeated course.[2] So in our project we aim to carried out the investigation of the putative role of ethanolic Extract of *Vernonia cinerea* in chemotherapy induced peripheral neuropathic pain in Mice . Based on the aim the objective may be conjured up as establish oxidative stress as pivotal factor under pinning pathology, Evaluating the damaging potential of oxidative

stress in chemotherapy induced peripheral neuropathic pain , Establishment of baseline characteristics by neuromotor and neurobehavioural profiling for selection of colony and testing of ameliorative potential of test drug in chemotherapy induced peripheral neuropathic pain in Mice .[3]

Aetiopathogenesis:

Epidemoiologically neuropathic pain has a greater prevalence in the global prospective .So we decided to narrowing down on neuropathic pain in our work ahead. As suggested in the literature the course of the neuropathic pain is often complicated due to the multifactorial etiology under pinning the conditions which demands closer overview . The pivotal contours may be featured as pain signaling changes, ion channel alteration, second ordered nociceptive neuronal alteration and environmental factor.

Pain signaling changes: Peripheral neuropathy alter the electrical properties of sensory nerves which leads to imbalance between central excitatory and inhibitory signaling .

Ion Channel Alteration: Neuropathy cause alteration ion channel such as Na^+ , K^+ and Ca^{2+} within the affected nerve which include all types of afferent fibers thus affecting the spinal and brain sensory signaling .

Second ordered nociceptive neuron alteration: It steams from altered sensitivity in second order neuron .Culminating in thalamus which lead to altered sensory transmission in the tertiary fibers sending impulse to cortex.

Environmental factors: Irregular life style such as smoking, drinking etc are the responsible for neuropathic pain .[4,5]

Modern Treatment Strategy: A Lacunae Revisited

Coming to the option available for management they include both non pharmacological approach and pharmacological approach . Under the non-pharmacological approach life style changes like monitored dieting , exercise may plays desire role in the therapy and sleep retardation therapy are deployed to control the functional aspect of the disease . On the other hand Pregabaline and Gabapentin are still considered as a corner stone of the therapy as per as pharmacological aspects . Pregabalin and Gabapetin both are bind with α2 and delta subunit resulting in decreased central sensitization and nociceptive transmission. Pregabalin is not effective because of starting dose of 150 mg/day,segregated either two or more times daily, that's why it's may be titrated up to 300 mg/day after one to two weeks. Since, in the modern era Pregabalin having some limitation. It's associated with Dizziness, Weakness Headache. On the other hand Gabapentin which is a Gold Standard Medication (GSM) started at a dose of 300 mg/day .However , Gabapentin are associated with Sedation ,ataxia and fatigue .Tricyclic antidepressant namely Amitriptyline , Nortryptyline are plays a pivotal role in the treatment of peripheral neuropathic pain . Tricyclic antidepressant are associated with cardiotoxicity . TCA should be started at low doses 10 mg to 25 mg /day at night and can be titrated up to 75 mg/day. Since serotonin norepinephrine reuptake inhibitors namely Duloxetine are plays a pivotal role in the treatment of peripheral neuropathic pain. Duloxetine block the presynaptic serotonin and norepinephrine transporters proteins. Duloxetine having some limitation it's associated with nausea,vomiting.Briefly, opioid like drugs that's are not recommended as first line therapy in the treatment of peripheral neuropathic pain. Opioid associated overdose, morbidity and mortality. Tramadol and Tapentadol are plays a crucial role in the treatment of peripheral neuropathic pain .Tapentadol is centrally acting opioid analgesic .Local anaesthetics having plays a putative role in the treatment of peripheral neuropathic pain namely Lidocaine , Pilocaine etc as per as pharmacological aspects .They blocks voltage gated sodium channel. However, it's having some limitations. Its act on the vital organ of the tissue that's why increase the chance to damage the vital organ namely Heart, Liver ,Lungs.[6,7,8]

***Vernonia cinerea*: A Brief Overview**

Vernonia is a genus of about 1000 species of forbs & shrubs in the family Asteraceae . Some species are known as ironweed .Some species are edible and of economical value . An erect , rarely decumbent , annual herb grows up to 75 cm in height . Steam : slender , grooved and ribbed . Leaves : simple , alternate , variable in shape , broadly elliptic or lanceolate , membranous or coriaceous. *Vernonia .c* is available on India , Bangladesh , Pakistan . This plant contains luteolin -7 mono beta D- glucopyranoside along with triterpene compounds like beta amyrin acetate , lupeol acetate . The sterol beta sitosterol , stigmasterol and alpha – spinasterol are present .

Extraction:

The fresh whole plant of *Vernonia cinerea* was shade dried at room temperature and reduced to coarse powder (Sieve no. 10/40) .The dried powder plant material of *Vernonia cinerea* (500g) was defatted with petroleum ether and then extracted with ethanol (95%) in a Soxhlet apparatus. Ethanolic extract of *Vernonia cinerea* was concentrated under reduced pressure to dryness (yield 15.12% w/w) .The purity of flavonoids in *Vernonia cinerea* was analyzed by High Performance Liquid Chromatography with VWD (Agilent Technologies 1220 infinity LC USA –Variable wavelength UV detector) Apigenin and Kaemferol were used as (external standard) marker compounds. The chromatography analysis was performed on a C18 column (4.6 x 150 mm) Mobile phase comprised of solvent A and solvent B and these two solvents were used with a constant flow rate of 1.0 ml/min .Solvent A consisted of 19% aetonitrile ,5% methanol and 1% THF in water (pH 3.0) , solvent B included 55% acetonitrile and 15 % methanol in water (Ph 3.0) The 20µl of *Vernonia cinerea* was injected into HPLC column and detection was performed at 352nm according to the standard operating procedure (Oncina et al . 2000) The retention time and spectrum of the *Vernonia cinerea* were compared with specific compound.

Determination of Total Phenolic Content:

The total phenolic content of *Vernonia cinerea* was determined by spectrophotometric method .(Harborne 1980 , Siddique et al. 2010) Approximately , 1 ml (0.5 and 1 mg/ml in ethanol) of ethanolic extracts of *Vernonia cinerea* was separately mixed with 0.5 ml of Folin Ciocalteu reagent (1N) and allowed to stand for 15 minutes. Then 1 ml of 10 % sodium carbonate solution was added to the above solution .Finally the mixture were made up to 10 ml with distilled water and the mixture of extract and reagent incubated at room temperature for 30 minutes .The absorbance of the reaction mixture was measured at 760 nm spectrophotometrically .Gallic acid was used as a reference standard and it was prepared in a variable concentration range that is 0 , 2 , 4 , 6 , 8 and 10µg/ ml of ethanol . The reaction mixture without sample was used as blank . Total phenolic content of ethanolic extract of *Vernonia cinerea* was expressed in terms of mg of gallic acid equivalent per gram of extract .[9.10]

Determination of Total Flavonoid content:

The assay was performed based on the process described previously by Harborne et al . in the year of 1980 with slight modification .Ethanolic extract Vernonia cinerea (0.5 ml of 1:10 g/ml in ethanol) was separately mixed with 1.5 ml of ethanol , 0.1 ml of 10 % w/v aluminium chloride , 0.1 ml of 1 M potassium acetate & 2.8 ml of distilled water .The mixture of extract and reagent were incubated at room temperature for 30 min ; the absorbance of the reaction mixture was measured at 415 nm. Quercetin was used as a reference standard and the quercetin was prepared in a variable concentration range i.e 0 , 10 , 20 , 30 , 40 and 50 µg/ml of ethanol. The content of total flavonoids was was expressed as quercetin equivalents (mg of quercetin equivalents / g of *Vernonia cinerea* extract)

METHODS

Fresh plant of *Vernonia cinerea* were collected from New Jalpaiguri , in the month of 3rd January .Plants were identified and confirmed by Mr. Subhomoy Panda , Department of Life Science .All the animals used in the study were taken care of under ethical consideration as per CPCSEA guidelines. The study was conducted after getting approval from Institutional Animal Ethics Committee (IAEC), Gupta College of Technological Sciences, Asansol(Protocol no: GCTS/IAEC/ 18 th December /04).So here is the blue print of the procedural aspects we have followed in our scientific research it start with male mice of age 8 weeks are chosen. They are assigned into different groups negative control group provided with normal saline, positive control group provided with Paclitaxel, standard group are provided with Paclitaxel and Gabapentin 5 mg/kg Test(1) group are provided with ethanolic extract of *Vernonia cinerea* 100mg/kg whereas Test(2) group are provided with ethanolic extract of *Vernonia cinerea* 200 mg/kg then we evaluate different parameters such as neurosensory paradigms , neuromotor paradigms , TNF-α assessment , Oxidative damage assessment

Fig1: Animal Husbandry

Inclined Plane Apparatus:

The method was deiscovered previously by Allmark and Bachinski et al in the year of 1949 with slight modification . The inclined plane is a 28 x 30 cm floor covered with a groved , 1 mm thick rubber surface and 20 x 30 cm walls 10 cm high on three sides . This task evaluates the animal's ability to maintain its body position by griping the edge of the plane by its front paw on a board that is incrementally raised to increasing angles . Animals are assigned into different groups then placed into the apparatus to take the baseline . The calculated amount of test drug was injected i.p to test animal and control group was injected only with vehicle as a same dose. After 30 mins periods the animals were placed on the inclined plane apparatus for association .[11]

Foot Fault Apparatus :

The assay was performed based on the process described by Macbrid et al. in the year of 2011 with slight modification .Animals were taken respectively bins and weights .They are assigned into two different groups respectively bin . Animals of all the groups dosed according to protocol .Then faulty step paradigm evaluated according to the standards.[12]

Fig 2: Foot Fault Test

Oxidative Damage Assessment:

The assay was performed based on the process described previously by Shrikant Atreya et al in the year of 2016 with slight modification .Tissue was taken then its homogenized .To 1.0 mL of the suspension 10% TCA was added. Then centrifuged was done for 5 mins at 5000 rpm .To 1.0 mL of supernatant , 1.0 mL of 0.67% TBA in 0.05% mol/L NaOH was added .Tubes were kept in boiling water bath for 20 min at temperature greater than 90 degree C and cooled. Then absorbance was measured at 532 nm spectrophotometrically .The concentration was measured with respect to blank was measured µg/ml .[13,14]

Statistical Analysis:

Results have been expressed as mean ± SD. One way ANOVA have been employed for comparing majority of parameters such as SOD, reduced GSH. Post hoc tests were used for identification of groups having significant differences for one way ANOVA. Tukey's Multiple Range Test was used for comparisons. Whereas for two way ANOVA Bonferronies test was used for the post hoc analysis .The significant groups were identified on the figure by designated alphabets.

RESULTS

Inclined Plane Test					
Grip Strength (angle of fall)	Negative control	Positive control	Standard	Test(1)	Test(2)
	75±0.08	50±1.03	67±1.01	70±0.9	72±1.02

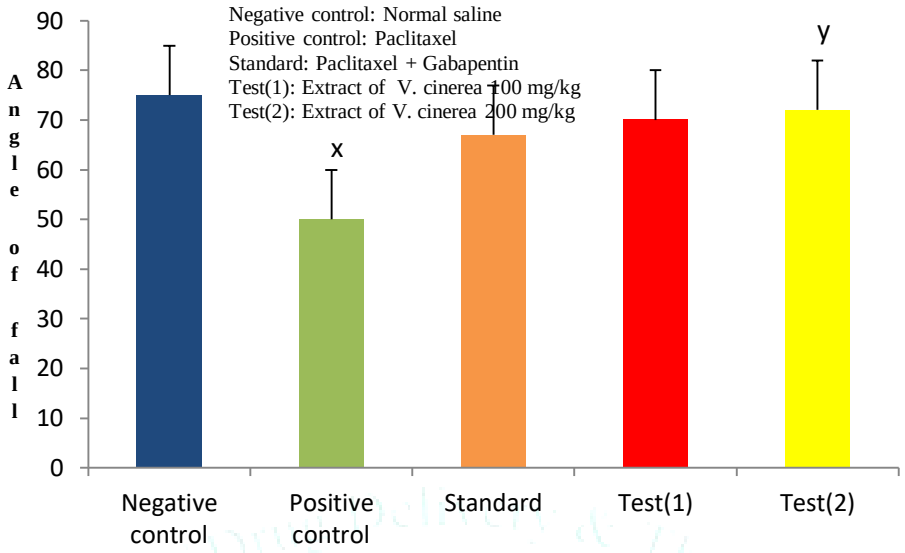

Fig 3 : Alteration of angle of fall in different group . Values presented as mean ± SD , ^{x} $p < 0.05$ in comparison between positive control and negative control group ^{y} $p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of angel a compare to negative control while the standard and test group showed an appreciable increase . However the most significant increase is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

Foot Fault Test					
Faulty step paradigms	Negative control	Positive control	Standard	Test(1)	Test(2)
	3	9	6	5	4

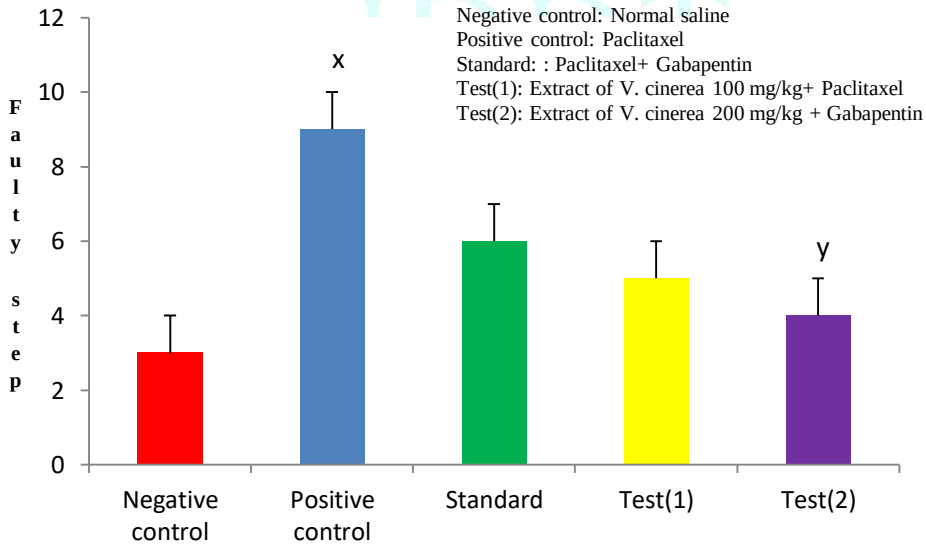

Fig 4: Variation of faulty step in different group. Values presented as mean ± SD , ^{x} $p < 0.05$ in comparison between positive control and negative control group ^{y} $p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of faulty step a compare to negative control while the standard and test group showed an appreciable decrease . However the most significant decrease is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

Differential TNF-α Expression in different Groups

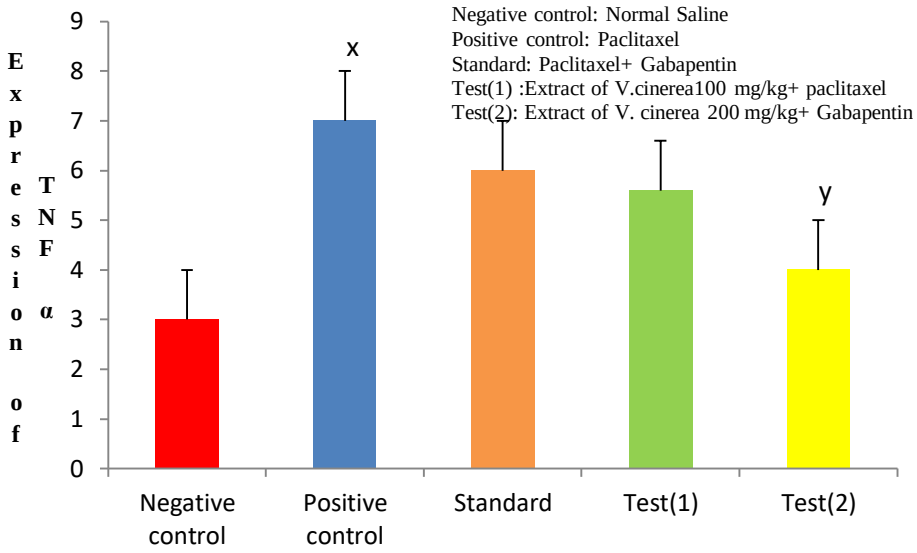

Fig 5: Differential TNF –α expression in different groups . Values presented as mean ± SD , ^{x} $p < 0.05$ in comparison between positive control and negative control group ^{y} $p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable decrease . However the most significant decrease is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

Differential TNF-α Inhibition Across Groups

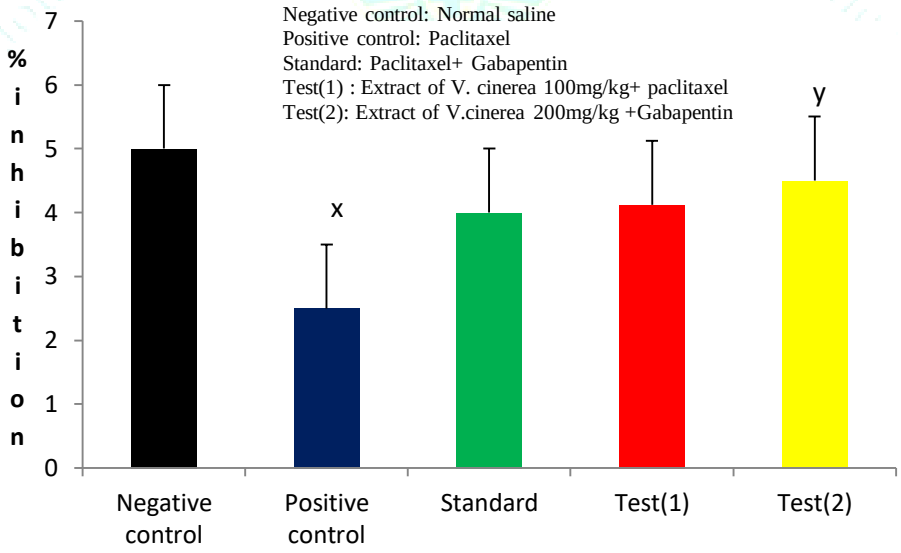

Fig 6: Differential TNF –α inhibition of different groups . Values presented as mean ± SD , ^{x} $p < 0.05$ in comparison between positive control and negative control group ^{y} $p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable decrease . However the most significant decrease is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

MDA Assessment:

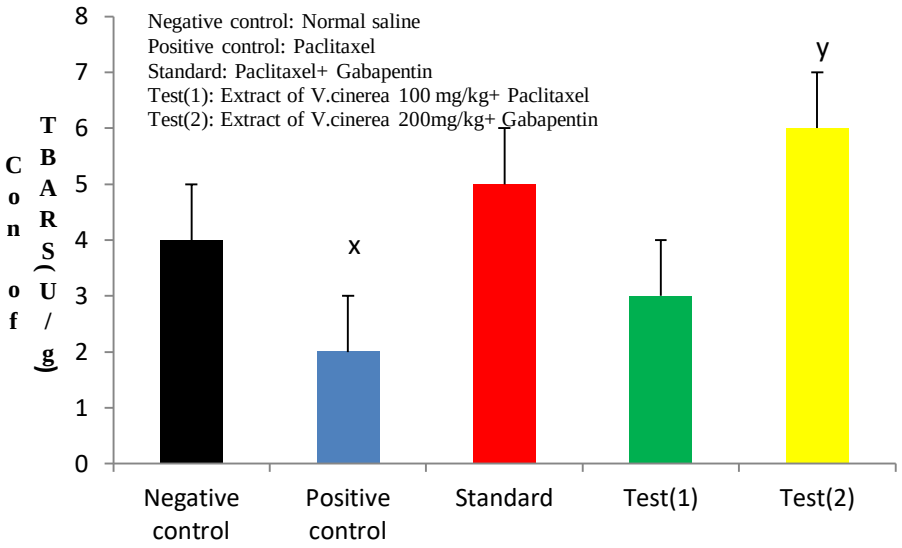

Fig 7: Variances in level of MDA in control and treated groups . Values presented as mean ± SD , $^{x}p < 0.05$ in comparison between positive control and negative control group $^{y}p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable decrease . However the most significant decrease is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

GSH Assessment:

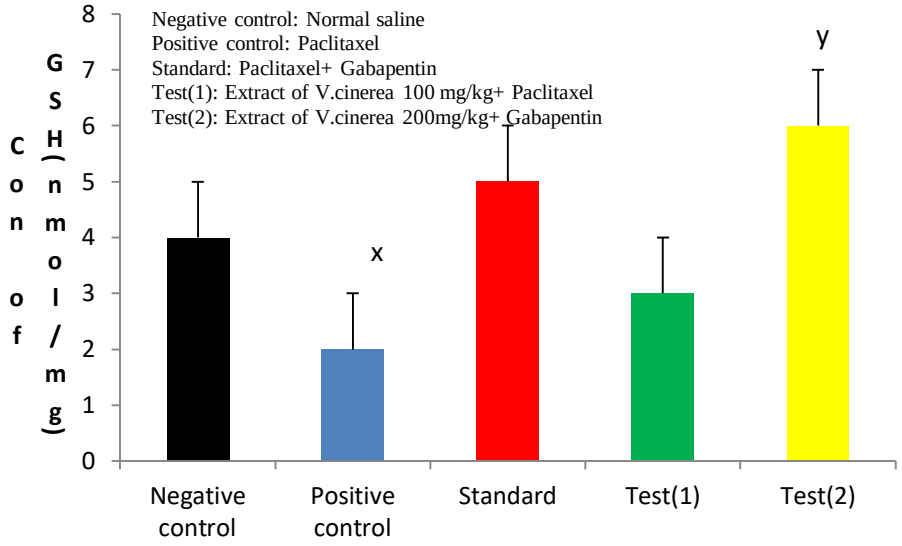

Fig 8: Variances in level of reduced GSH in control and treated groups . Values presented as mean ± SD , $^{x}p < 0.05$ in comparison between positive control and negative control group $^{y}p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable increase . However the most significant increase is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

SOD Assessment

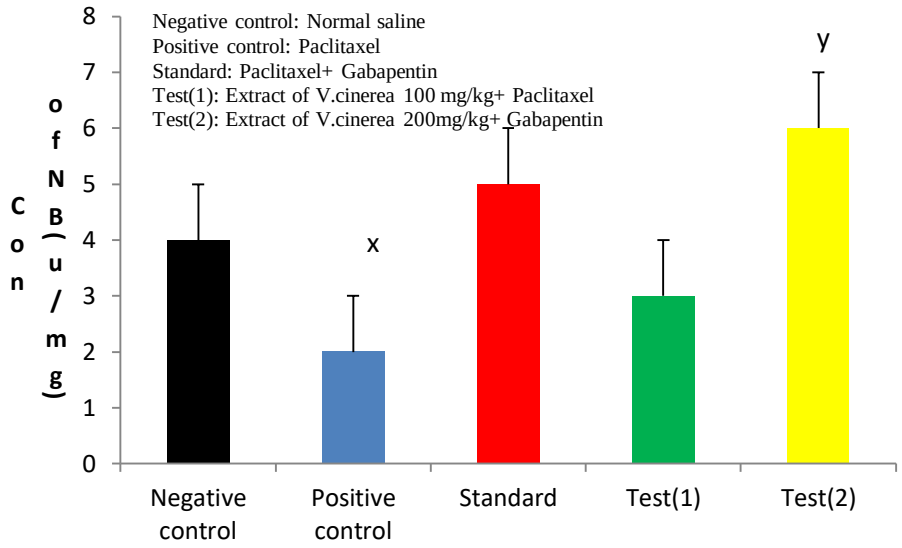

Fig 9: Variances in level of SOD in control and treated groups . Values presented as mean ± SD , $^{x}p < 0.05$ in comparison between positive control and negative control group $^{y}p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable increase . However the most significant increase is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

Catalase Assessment:

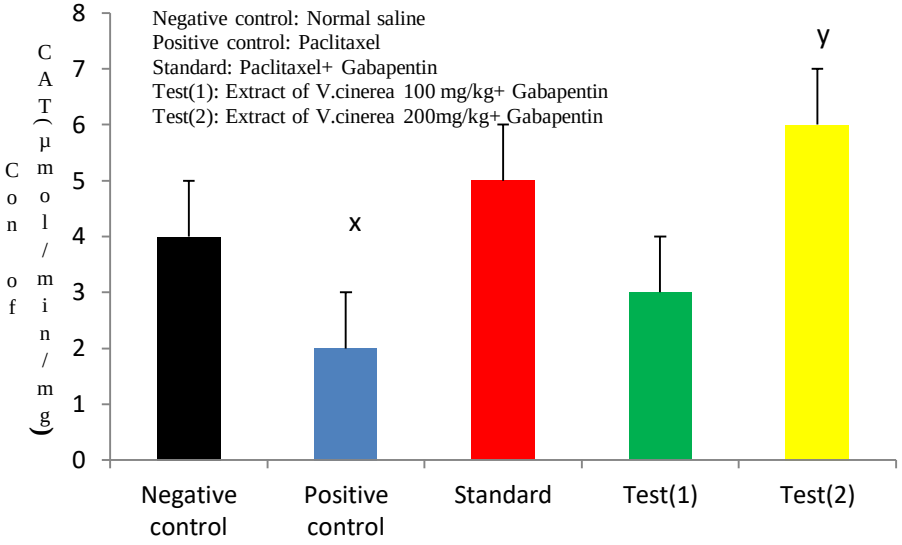

Fig 10 : Variances in level of CAT in control and treated groups . Values presented as mean ± SD , $^{x}p < 0.05$ in comparison between positive control and negative control group $^{y}p < 0.05$ in comparison between positive control and treated group .

Positive control group treated with paclitaxel was found to have a significant hike in level of nerve tissue a compare to negative control while the standard and test group showed an appreciable increase . However the most significant increase is observed in Test(2) group which is treated with ethanolic extract of *V. cinerea* 200 mg/kg .

DISCUSSION

As we know neuropathic pain is an age old menace .According to International Association for Study of Pain is defined as pain initiated or caused by injury or dysfunction of the somatosensory pathway. The injury or dysfunction may involve peripheral or central nervous system structures characterized by pain, numbness tingling in the extremities and slow nerve conduction .Epidemiologically neuropathic pain has a greater prevalence in the global prospectives .Hence, we decided to narrowing down peripheral neuropathic pain in our work ahead. As suggested in the literature the course of neuropathic pain is often complicated due to the multifactorial etiology under pinning the condition which demands closer overview. The pivotal contours may be featured as pain signaling changes, , ion channel alteration and second ordered nociceptive neuronal alteration .

Coming to the option available for management they include both non pharmacological aspects and pharmacological aspects. Under non pharmacological aspects life style changes like monitored dieting, exercise plays a pivotal role in the treatment of peripheral neuropathic pain. On the other hand Gabapentin and Pregabalin plays a crucial role as per as pharmacological aspects.

However, Gapbapentin and Pregabalin have some limitation. Gabapentin associated with sedation and Pregabaline associated with dizziness. Hence we found a good rationale behind probing for prophylaxis role in our pipeline that is *Vernonia cinerea*

So in our study we take male mice of age 8 weeks and they are divided into different groups such as Negative control treated with normal saline where as positive control treated with paclitaxel standard provided with paclitaxel and pregabaline on the other hand Test(1) provided with extract of *vernonia cinerea 100 mg/kg* and Test (2) provided with extract of *Vernonia cinerea* 200 mg/kg as per protocol .

The severity of peripheral pain in the disease was evaluated by measuring standard parameters namely neurosensory assessment, MDA, reduced GSH activity , TNF-α Assessment. In present study , we found that Test(1) increased the fletching threshold in the following treatment with ethanolic extracts of *Vernonia cinerea* 200 mg /kg significantly declined the scored compared to the positive control group .This is the line with the conclusion published by Koryea et al in the year of 2016 .

Many studies have revealed that the increase of oxidative stress MDA and GSH has been notable feature of Peripheral Neuropathic Pain , which resulted in a pathological cascade of free radical reactions and further yielding more oxidative free radicals .Failure of the endogenous antioxidant defense mechanism promote formation of excessive free radicals and consequent tissue damage .Parameters such as MDA , GSH activity can be indicative of oxidative stress status of the disease .MDA level can be determining by TBARS .As observation in our study increase in the MDA levels in the periphery affected by the paclitaxel administration suggests enhanced lipid peroxidation that could be responsible for the tissue damage . This is the line with the conclusion published by Macbrid et al. in the year of 2017.

In the present study animal group treated with paclitaxel suffered an increase in the oxidative stress indicated by higher MDA and GSH activity which are responsible for the tissue damage and amelioration of pain respectively bin. In our study we found that animals treated with extract of *Vernonia cinerea* had reduced MDA expression and increased GSH activity which was significant in comparison to the positive control group thus suggesting its antioxidant property.

CONCLUSION

As we know neuropathic pain is an age old menace . So , here we concluded that ethanolic extract of *Vernonia cinerea (200 mg/kg)* have a putative role in Chemotherapy induced neuropathic pain in Mice .

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