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RESEARCH ARTICLE

PROTECTIVE EFFECT OF PHENOLIC EXTRACT OF *CUCURBITA PEPO* SEEDS IN NEUROPATHIC PAIN MODELS

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Abstract:

Neuropathic pain arising from peripheral nerve injury is a clinical disorder characterized by a combination of spontaneous pain, hyperalgesia and tactile pain (allodynia), and remains a significant clinical problem. It is a complex clinical syndrome which affects large number of population in the world, has a massive cost for the health care system and is personally devastating to the people who experience it. In the present study the extraction of phenolic compounds from the seeds of *Cucurbita pepo* was done where vanillin as a phenolic compound is mainly focused in the study which was used for evaluating neuropathic pain management activity using pyridoxine induced neuropathic pain and alcohol induced neuropathic pain models. The main objective was to explore the analgesic activity of extract by checking the tail flick latency and inclined screen performances of rats in pyridoxine induced model and also to determine the antioxidant activity of extract by estimation of TBARSs and glutathione assay in sciatic nerves of alcohol induced neuropathy in rats. It was found that phenolic extract of c.pepo seeds showed a significant antineuropathic pain activity.

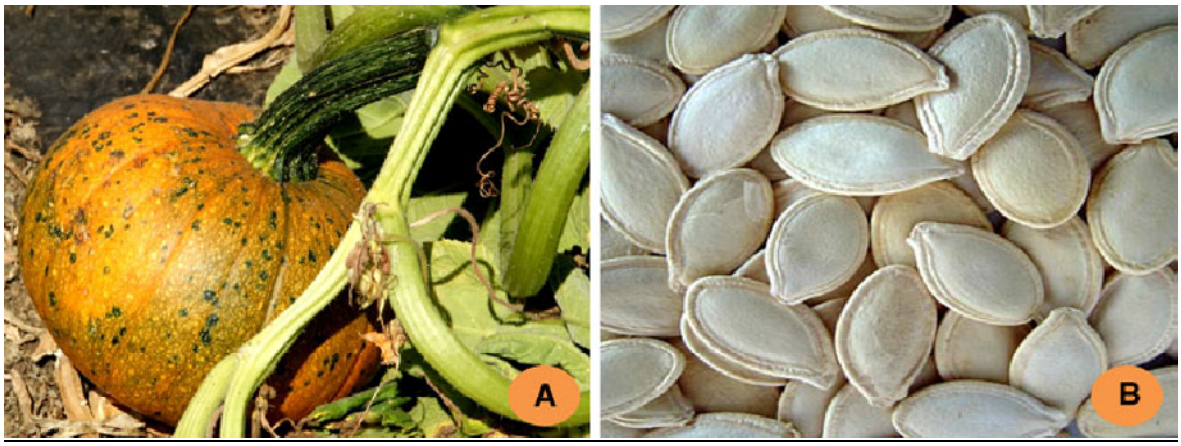
Key Words: Neuropathic pain, hyperalgesia, allodynia, *Cucurbita pepo*, phenolic extract, antioxidant activity.

INTRODUCTION:

Neuropathic pain is defined as "pain initiated or caused by a lesion or dysfunction in the peripheral or central nervous system"[1].Neuropathic pain is persistent (as opposed to transient) and appears to serve no biological function. It is the phenomenon of stimulus-independent or spontaneous pain that distinguishes neuropathic pain from physiological pain. Spontaneous pain, as its name suggests, takes place in the apparent absence of any stimuli. Possible reasons for the production of stimulus independent pain are spontaneous activity of the A and C fibers and/or the formation of sympathetic baskets around the large sized neurons in the DRG[2]. Neuropathic pain can manifest itself as either without a stimulus (stimulus-independent pain) and/ or as pain hypersensitivity elicited after a stimulus (stimulus-evoked pain). Stimulus-independent pain includes symptoms described by the patient such as (a) continuous burning pain (b) intermittent shooting, lancinating pain (c) some dysaesthesias. Conversely, stimulus-evoked pain describes signs that physician induces after mechanical, thermal or chemical stimulation, and usually involves hyperalgesia or allodynia[3].

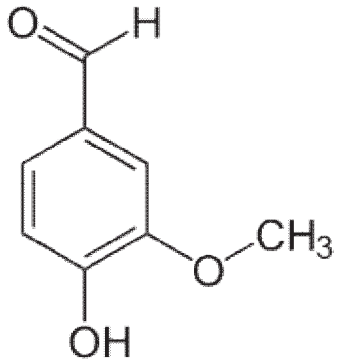
Neuropathic pain often seems to have no obvious cause; but some common cause of neuropathic pain include:- Excessive Alcoholism, Amputation, Back-leg and hip problems, Diabetes, Facial nerve problems, HIV infection or AIDS, Multiple sclerosis Shingles, Spine surgery. Neuropathic pain is very challenging to manage because of the heterogeneity of its aetiologies, symptoms and underlying mechanisms[4]. In addition to their potential benefits, all of drug classes are associated with various adverse effects. Thus, the idea of treating pain with different herbs including various constituents is particularly less toxic and has better effect[5].

Cucurbita Pepo (common name: Pumpkin seed, kaddu ke beej. Bhopala bij) is an annual plant, hispid and scabrous, with a procumbent stem and branching tendrils. The seeds are "about 2 Cm. (4/5 inch) long, broadly-ovate, flat, white or whitish, nearly smooth, with a shallow groove parallel to the edge; containing a short, conical radicle, and 2 flat cotyledons; inodorous; taste bland and oily"[6].



Pumpkin seed is traditionally used to treat a wide variety of illnesses, and through scientific investigation most of the properties has been validated such as antidiabetic, antiarthritic, cancer management, antispasmodic.etc[7].

Phenolic compounds are chemically classified into many classes such as tannins, flavonoids, coumarins and lignans. Tannins represent the largest group of polyphenols, they are widely distributed in the bark of trees, leaves, stem and fruits measured the total phenolics content in the pumpkin seed oil, which ranged from 25 to 51/mg GAE/kg of oil. The individual phenolics in pumpkin seeds were tyrosol, vanillic acid, vanillin, luteolin and sinapic acid. The maximum antioxidant capacity measured by the reduction of the DPPH radical[10, 11,13].



VANILLIN (Phenolic comound)

It is believed that the high level of vanillin intake from foods and beverages will have beneficial effects on human health. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is known to have antimutagenic, anti-invasive, and metastatic suppression potential. Antinociceptive property in acetic acid and antioxidant and hepatoprotective properties in carbon tetrachloride treated rats have also been demonstrated[8, 11,12].

Pregabalin was selected as a standard drug for the study, which is an anticonvulsant use in treatment of neuropathic pain (pain in damaged nerves). It acts by blocking voltage gated calcium channels and hence decreases glutamate and sensory neuropeptides release, thus reduces the activation of NMDA receptors and decreases neuronal firing, thereby reducing pain. It has been shown that 2mg/kg intravenous administration of pregabalin through tail vein of rats shows antiallodynic and antihyperalgesic effects.

MATERIALS AND METHOD:

Phenolic compounds were isolated from *Cucurbita pepo* seeds by using organic and inorganic solvents[9].

Fifty grams of seeds ground was mixed with 200 ml of hydrochloric acid (2%) and the mixture was placed in waterbath for one hour at 90°C.The mixture was then stirred on magnetic stirrer for two hours. Filtration was achieved by using Buchner funnel, the filtrate was treated with 200 ml of diethylether with the same volume of filtrate. The mixture was put other time in water bath for one hour, then it was evaporated by using rotary evaporator and finally crude phenols were obtained

Phytochemical screening:

The phenolic compounds which were isolated were underwent to many detections such as[10]:

1. Phenolic Compounds Detection: To 2-3 ml of test solution added few drops of following reagents and was observed for respective coloration or precipitate.

1. 5% Ferric Chloride Solutions: Deep blue-black coloured

2.Flavonoids Detection:

1. Shinoda Test: To the test solution added 5 ml of 95% ethanol, few drops of concentrated HCL and 0.5 gm of magnesium turnings. Pink color was observed.

3. Tannins Detection:

Ferric chloride test: A few drops of 1% neutral ferric chloride solution were added to the solution of the extract, and observation was recorded.

4.Carbohydrates Detection:

Molisch's test: To 2-3 ml of test solution, few drops of alcoholic alpha napthol solution were added and shaken, Concentrated H_2SO_4 was added from the side of the test tube, formation of violet ring at the junction of two layers was observed.

5.Alkaloids Detection: To small amount of Phenolic extract, shaked well and filtered. Filtrate was collected and following tests were performed.

1. Dragendroff's test: To 2-3 ml filtrate added few drops of Dragendroff's reagent and was observed for orange brown precipitate.
2. Hager's test: To 2-3 ml of filtrate added few drops of Hager's reagent and observed for yellow precipitate.

6.Saponin Detection:

Foam test: Small amount of extract was shaken with little quantity of water, and the observation was recorded.

7.Glycosides Detection:

Keller –Killiani test**:** 2 ml of the extract, 3 ml of glacial acetic acid and 1 drop of 5 % ferric chloride was added. This solution was carefully transferred to the surface of 2 ml conc. H2SO4 and the observation was noted down.

THIN LAYER CHROMATOGRAPHIC ANALYSIS:

TLC technique was depended for separation of phenolic compounds present in phenolic extract and determination of their purity. The standards and phenolic extract of C.pepo sample solutions were applied to the TLC plates. The plates were developed with a mobile phase of Methanol: Water: Glacial acetic acid [20:05:02] in a TLC twin trough chamber. After development the plates were dried at 60˚C for 5 minutes and the quantification of the standards and samples was done under UV light[11,12].

PRELIMINARY TOXICOLOGICAL EVALUATION

Animal House Conditions:

Wistar rats of 200-250 gms were purchased from Bharath serums and vaccines Limited, Plot no. A-371-372, Road NO.27,Wagle Industrial Estate, Thane 400604, Maharashtra, Registration no. 103/99/CPCSEA. The animals were brought to animal house of L.H. Hiranandani college of Pharmacy, opposite to Ulhasnagar station, CHM Campus, Ulhasnagar-03. These animals were acclimatized in animal house of Dr under standard husbandry conditions, i.e.; room temperature of 24 ± 10 C; relative humidity 45-55% and a

12:12hr light/ dark cycle. The institution's animal house is registered with Govt. of India, having registration no 103/99/CPCSEA.

Acute toxicity study of test drug was performed according to OECD guidelines 423. Animals were fasted overnight and then test substance (Phenolic extract of *Cucurbita pepo*) was provided with single oral dose of 2000mg/kg. Rats were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter up to 14 days. The parameters observed were: grooming, hyperactivity, sedation and diarrhea, loss of righting reflex, convulsion and death.

IN VITRO ANTIOXIDANT ACTIVITY:

To determine the anti-oxidant activity of phenolic extract of C.pepo.

DDPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity:

The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Extract reduces the colour of DPPH due to the power of hydrogen donating ability. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. The free radical scavenging activity of the extracts and Quercetine as positive control was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 5 different concentrations were prepared for test and control (20ug/ml, 40ug/ml, 60ug/ml, 80ug/ml and 100ug/ml). Freshly DPPH solution was prepared in methanol. Reaction mixture was allowed to stand for 30 mins and absorbance was taken at 517nm. The percentage inhibition of DPPH free radical scavenging activity and IC50 was calculated[13].

- **% Inhibition :**

  (Abs Control – Abs Test)/ Abs Control x 100.

INVIVO NEUROPATHIC PAIN MODEL:

Pyridoxine Induced neuropathic pain:

Vitamin B6 is a dietary requirement of non-ruminant animals and is a coenzyme in many important biological reactions. Pyridoxine intoxication associated sensory neuropathy in humans has been reproduced in different animal species Administration of higher doses of pyridoxine leads to the over expression of glutamate, which activates NMDA receptors. Thus hyperexcitability of NMDA receptors leads to generation of Neuropathic pain. Pyridoxine intoxicated rats develop a peripheral neuropathy characterized by sensory nerve conduction deficits associated with disturbances of nerve fiber geometry and axonal atrophy.

Procedure:

Male Wistar rats weighing 200-250 grams were divided into four groups of six animals each. Pregabalin served as the reference standard as anti-neuropathic drug. After one hour of the administration of the drugs, Pyridoxine 400mg/kg will be administered to rats intra-peritoneally, twice daily for a period of 14 days. Behavioural parameters will be assessed by tail flick test, incline screen performance on the $0,7^{th}$ and 14^{th} day after dosing the animals[14,15].

Ethanol induced neuropatic pain:

Chronic alcohol consumption produces painful peripheral neuropathy Alcoholic neuropathy involves coasting caused by damage to nerves that results from long term excessive drinking of alcohol and is characterized by spontaneous burning pain, hyperalgesia and allodynia. The mechanism behind alcoholic neuropathy is not well understood, but several explanations have been proposed. These include activation of spinal cord microglia after chronic alcohol consumption, oxidative stress leading to free radical damage to nerves, activation of mGlu5 receptors in the spinal cord and activation of the sympathoadrenal and hypothalamo-pituitary-adrenal (HPA) axis.

Procedure:

Male Wistar rats weighing 200-250 grams were divided into four groups of six animals each. Pregabalin served as the reference standard as anti-neuropathic drug. Alcoholic Neuropathy was induced by administration of 10g/kg of 35% v/v ethanol for 7 weeks. The dose of ethanol was decided on the basis of the existing literature. After one hour of the

administration of the drugs, ethanol is administered orally twice daily for the period of 7 weeks[16].

Density of absolute ethanol = 99.9% (v/v).

Desired Concentration =35% (v/v)

So to make 35% v/v ethanol, 35ml of absolute ethanol was added to 65ml of distilled water to make the volume up to 100 ml.

Calculation of Dosing Volume

Density of final mixture (35% v/v ethanol) = 0.97gm/ml.

Therefore, Administered Volume = Dose (10 g/kg) x Rat Body Weight /1000 x Density (0.97 g/ml).

Behavioral parameters will be assessed by Hot plate test, Tail immersion test weekly.

On the last day after dosing the animals and observing the behavioural changes, animals will be sacrifice and biochemical parameters will be assessed.

BIOCHEMICAL PARAMETERS:

Collection of tissue samples in rats

For biochemical assessment, at the end of treatment schedule, Sciatic nerves were rapidly removed, washed with sterile in normal saline and weighed. A 10% (w/v) tissue homogenate is prepared in 0.1 M phosphate buffer (pH 7.4) and centrifuged for 15 min at 2000 g to obtain the clear supernatant.

Estimation of lipid peroxidation:

Principle: Lipid peroxidation, a well-established mechanism of cellular injury in plants and animals, is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition, and the measurement of MDA and HAE has been used as an indicator of lipid peroxidation.

Procedure:

Concentration of thiobarbituric acid reactive substances (TBARS) was determined as an index of lipid peroxidation as described by the method of Niehius and Samuels. In this

method, 0.1 ml of supernatant of sciatic nerve homogenate was treated with 2 ml of (1:1:1 ratio) thiobarbituric acid-trichloroacetic acid-hydrochloric acid (TBA-TCA-HCL) reagent. TBARS reagent was prepared by mixing equal volumes of TBA (37%), TCA (15%) and HCL (0.25 N). Then the mixture was placed in hot water bath for 15 min, cooled and centrifuged at 1000 rpm for 10 mins. The absorbance of the clear supernatant was measured at 532 nm (UV-1700 Spectrophotometer) against blank. Finally, the values are expressed as nmole/mg of protein[17,18,21].

Estimation of reduced glutathione

Principle: Glutathione is currently one of the most studied antioxidants. This is likely due to it being endogenously synthesized all throughout the body and it is basically found in all cells, sometimes in rather high concentrations. In peripheral neuropathy the nerve fibres are damaged leading to the generation of oxidative stress and decrease in glutathione antioxidant activity.

Procedure:

The concentration of endogenous antioxidant reduced glutathione (GSH) level in the alcoholic neuropathy will be estimated .In this method, 1.0 ml of sciatic nerve homogenate (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation for 15 min. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1M, pH 7.4) and 0.2 ml 5,5, dithiobis (2- nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm and the reduced GSH levels were expressed as μmole/mg of protein in sciatic nerve[18,19,20].

RESULT AND DISCUSSION:

The present study dealt with phytochemical and pharmacological evaluation of the seeds of *Cucurbita pepo*. The phenolic extract of *Cucurbita pepo* seeds were evaluated for phytochemical and pharmacological characteristics, at dose of 400 mg/kg.

Table:1 Phytochemical test for different compounds in extract:

Sr.no	Test	Inference
1	phenols	+
2	flavonoids	+
3	alkaloids	+
4	carbohydrates	+
5	saponins	+

Identification by Thin Layer chromatography:

Rf value for Phenolic extract of C.pepo was 0.88 and standard Vanillin 0.88 which was similar to each other thus suggested the presence of Vanillin in extract.

Acute Toxicity Study:

The test drug was found safe at 2000mg/kg bodyweight. Slight sedation and grooming were observed for first 4 hrs. After 24 hrs all animals were observed normal when compared with control group. There was no death of animals during or after 14 days. The main purpose of this study was to identify safe therapeutic dose as well as to observe behavioral changes and toxicity. $1/5^{th}$ of maximum dose of test drug (Phenolic extract of C.pepo) used in acute toxicity testing was considered as maximum therapeutic dose for experiment.

In-vitro models of antioxidant activity (DPPH Free radical scavenging activity):

Phenolic extract of C.pepo showed a promising free radical scavenging effect on DPPH radical in a concentration dependant manner. The extract was compared with Quercetin which was used as a standard antioxidant. The IC50 value of extract was found to be 23.113 µg/ml and that of standard is 18.317 µg/ml. The correlation coefficient (R^2) was calculated from graph and was found to be o.9877 of extract and 0.992 of standard.

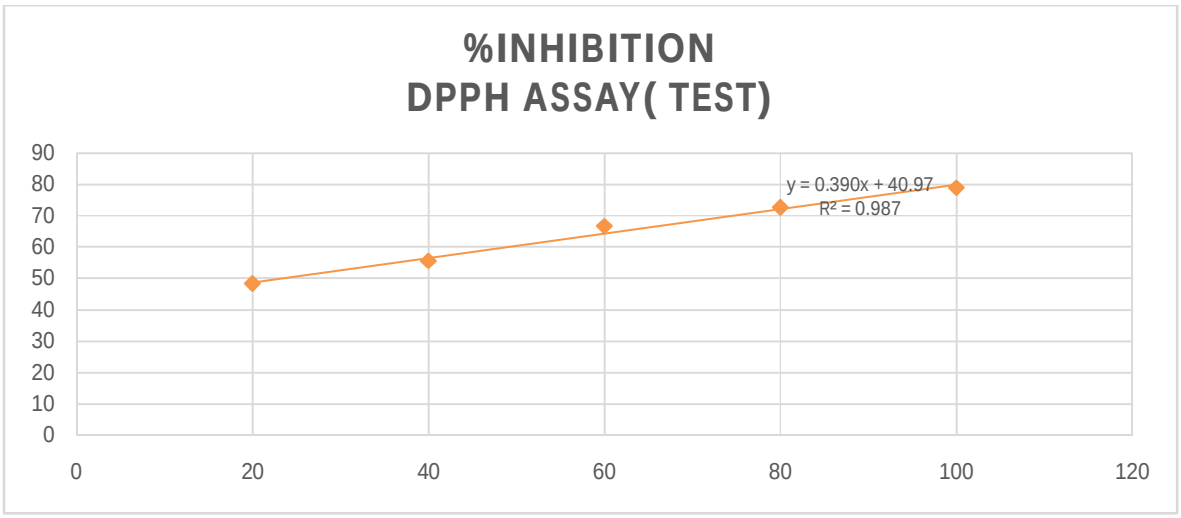


Figure 1. Radical scavengic activity of extract

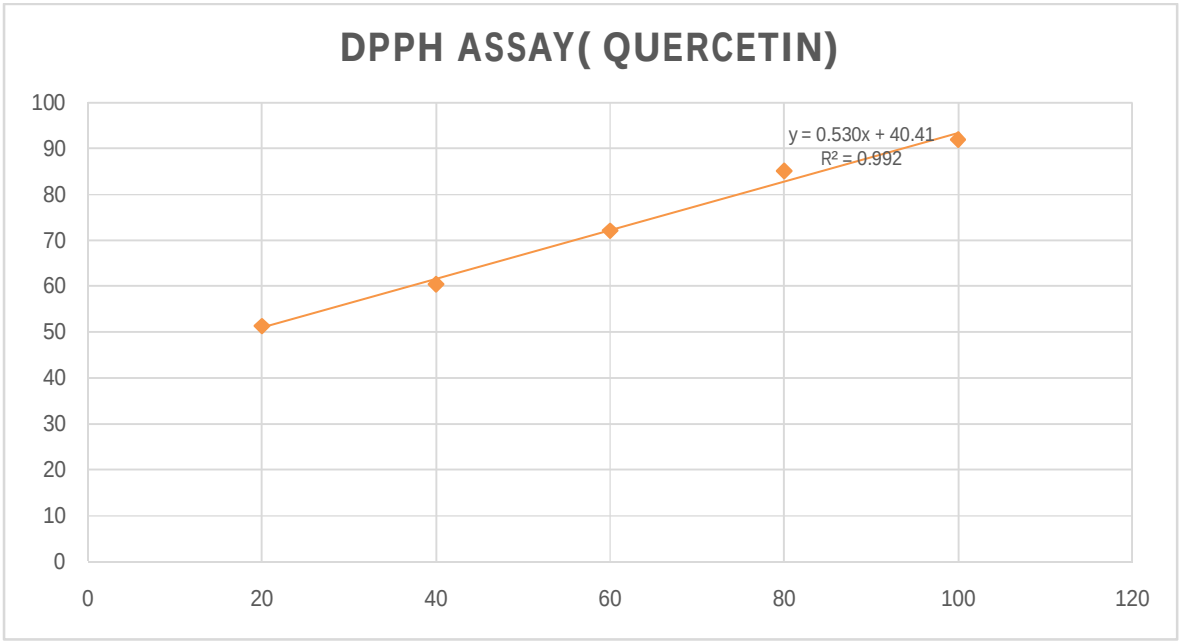


Figure .2. Radical scavenging activity of standard

IN-VIVO models for neuropathic pain:

Pyridoxine Induced Neuropathic Pain

In the present study the test group phenolic extract of C.pepo at dose level of 400mg/kg and positive control group pregabalin (2mg/kg i.v) produced significant ($p< 0.005$) antagonism of pyridoxine induced increase in the tail flick latency, and also produces significant increase in motor coordination and muscle strength activity when compared with pyridoxine induced neuropathy.

Table2:Tail flick latency:

Day/Treatment	Control	Disease Control	Standard	Test
Day 0	3.9 ±0.058	3.9 ±0.030	4.1 ± 0.018	3.9 ±0.02
Day 7	3.35± 0.056	7.3 ±0.045	5.02 ± 0.014	6.11±0.024
Day 14	3.98± 0.03	9.12 ±0.023	4.5 ± 0.016	5.34 ±0.01

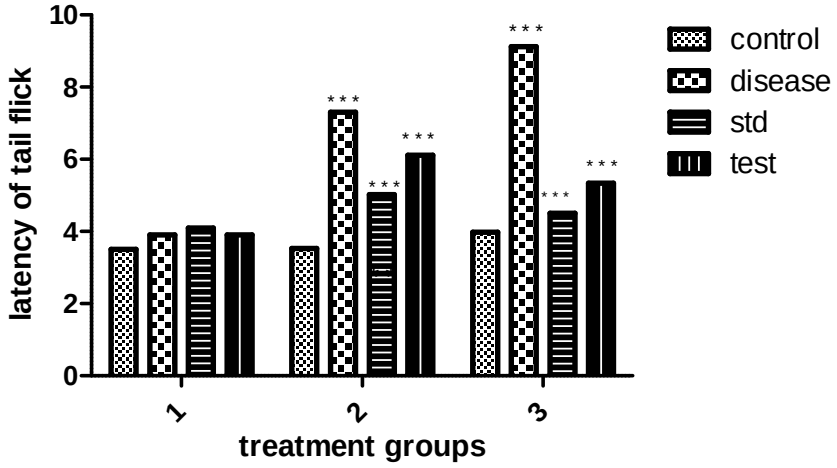


Figure 3: Tail flick latency

Table 4: Inclined screen performances:

Day/Treatment	Control	Induced	Standard	Test
Day 0	66.50 ±0.281	67.20±0.375	67.1 ±0.33	65.86±0.261
Day 7	66.30 ±0.21	44.15 ±0.133	59.40±0.184	53.40±0.286
Day 14	64.80±0.19	28.60 ±0.17	56.59±0.09	49.96±0.20

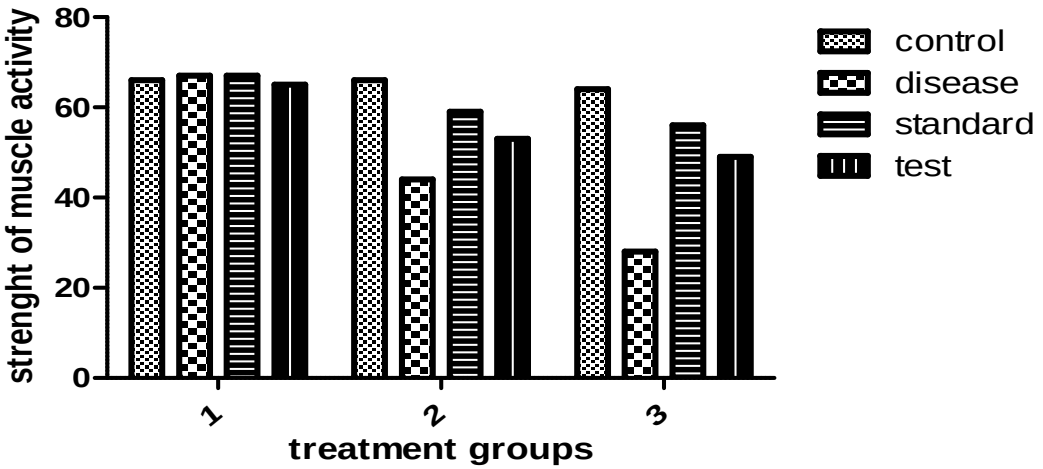


Figure:4 Inclined screen performances

Alcohol Induced neuropathic pain:

Estimation of hot plate test (Thermal hyperalgesia):

Ethanol administration, twice a day, for 7 weeks significantly decreased ($p< 0.005$) mean paw licking response threshold compared to normal control rats. Chronic treatment with the dose of *Phenolic extract of C.pepo (4*00 mg/kg) for 7 weeks significantly and dose dependently increased paw licking threshold in ethanol treated rats . Treatment with a positive control group pregabalin (2mg/kg, *i.v.*) significantly attenuated ethanol induced neuropathic pain as compared to ethanol control group.

Table 5: Estimation of hot plate method

Weeks/Treatment	Latency of paw licking and jumping from hot plate			
	Control	Inducing	standard	Test
Week 1	44.60±0.1687	45.90±0.1478	43.70±0.2167	46.90±0.2587
Week 2	44.60±0.1667	40.00±0.1909	42.00±0.1528	41.60±0.1054
Week 3	47.80±0.2777	33.50±0.1558	39.80±0.1606	37.89±0.1389
Week 4	47.80±0.2616	28.40±01498	34.70±0.2040	31.65±0.1377
Week 5	47.20±0.1978	22.72±0.1240	32.20±0.1606	28.40±0.1116
Week 6	46.50±0.1500	18.10±0.1167	30.60±0.1265	25.50±0.1147
Week 7	44.70±0.1176	9.950±0.1357	28.40±0.1202	23.20±0.1174

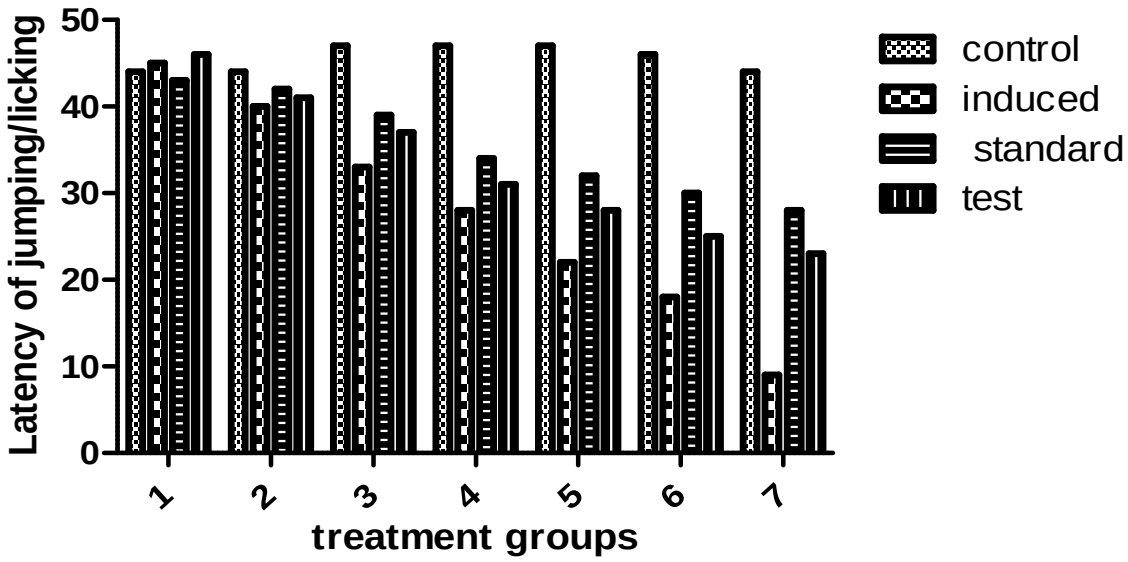


Figure 5: Estimation of thermal hyperalgesia

Estimation of tail immersion test (cold allodynia) :

Ethanol administration for 7 weeks significantly decreased ($p< 0.005$) mean tail withdrawal latency as compared to normal control rats. Significant and dose dependent restoration of decreased mean tail withdrawal latency was observed in rats treated with Phenolic *extract of C.pepo (4*00 mg/kg) as compared to ethanol treated rats. Treatment with positive control group pregabalin (2mg/kg, *i.v.*) significantly attenuated ethanol induced neuropathic pain as compared to ethanol control group.

Table 6: Estimation of cold allodynia

Week/ Treatment	Flicking response/sign of struggle in cold water (4^0c)			
	Control	**Inducing**	**Standard**	**Test**
Week 1	59.00±0.2216	60.50±0.2007	60.90±0.3284	59.90±0.1990
Week 2	58.10±0.1797	59.90±0.2040	59.90±0.2301	58.10±0.2007
Week 3	59.50±0.1641	40.90±0.1249	48.50±0.1701	44.25±0.1181
Week 4	59.70±0.1607	32.33±0.1753	46.90±0.1222	41.77±0.06274
Week 5	58.00±0.1406	23.06±0.1228	42.50±0.1537	37.91±0.1659
Week 6	58.30±0.1600	17.15±0.1158	39.70±0.1470	35.50±0.1851
Week 7	58.80±0.2129	11.77±0.08796	37.90±0.1424	33.80±0.1783

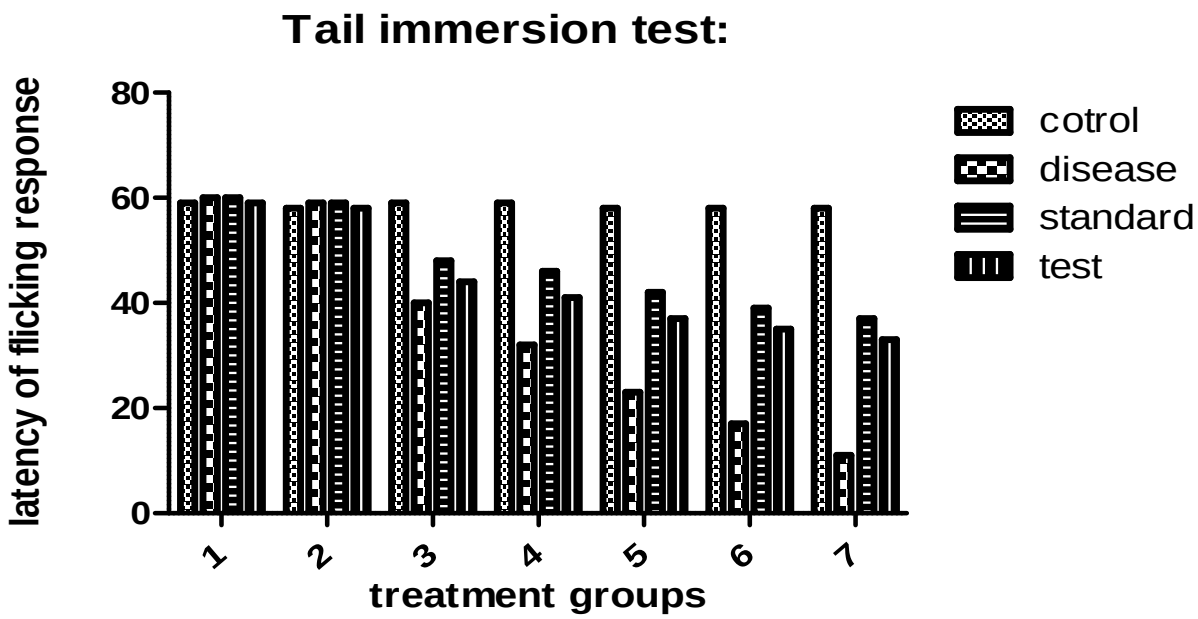


Figure 6: Estimation of cold allodynia

Estimation of lipid peroxidation:

Administration of Phenolic *extract of C.pepo* (400 mg/kg) significantly decreased TBARS level as compared to alcohol control rats. Treatment with positive control group pregabalin (2mg/kg, *i.v.)* significantly reduced the elevated level of TBARS as compared to ethanol control group.

Table 7: Estimation of TBAR assay

Groups	Treatments	Level of TBARS (nmole/mg)
Control	Distilled water	25.62±0.2755***
Disease control	Ethnanol	76.92±0.9098***
Standard	Pregabalin	39.90±0.4248***
Test	Phenolic extract of C.pepo	49.46±0.3248***

All values are expressed as mean ± SEM (n = 6); one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. P < 0.005 significance compared to disease control.

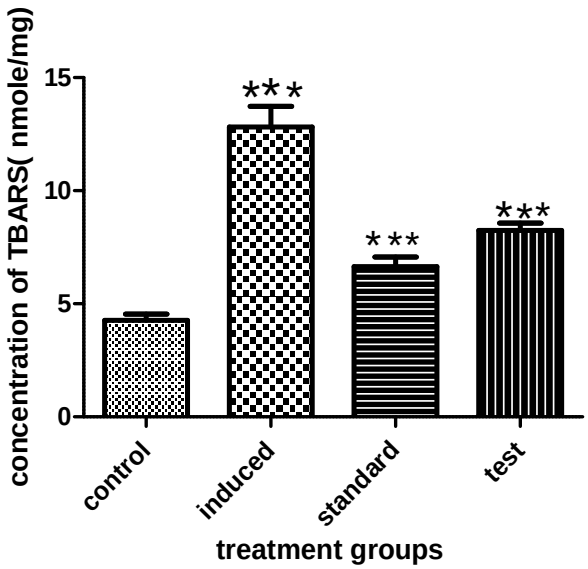


Figure 7: Estimation of TBAR assay

Reduced Glutathione assay:

Nerve injury causes marked reduction in GSH level in the sciatic nerve of ethanol treated rats. The reduced glutamate level was significantly decreased as compared to normal control rats. This GSH reduction was significantly (p< 0.005) and dose-dependently reversed by the administration of Phenolicextract *of C.pepo (4*00 mg/kg) in ethanol treated rats. Treatment

with pregabalin (2mg/kg, *i.v.)* significantly restored the depleted level of GSH as compared to ethanol control group.

Table 8: Estimation of reduced glutathione assay

Groups	Treatments	Concentration of glutathione (µmole/mg)
Control	Distilled water	3.420±0.02992
Disease Control	Ethanol	0.7508±0.0062
Standard	Pregabalin	2.624±0.03520
Test	Phenolic extract of C.pepo	2.052±0.03065

All values are expressed as mean ± SEM (n = 6); one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. P < 0.005 significance compared to disease control.

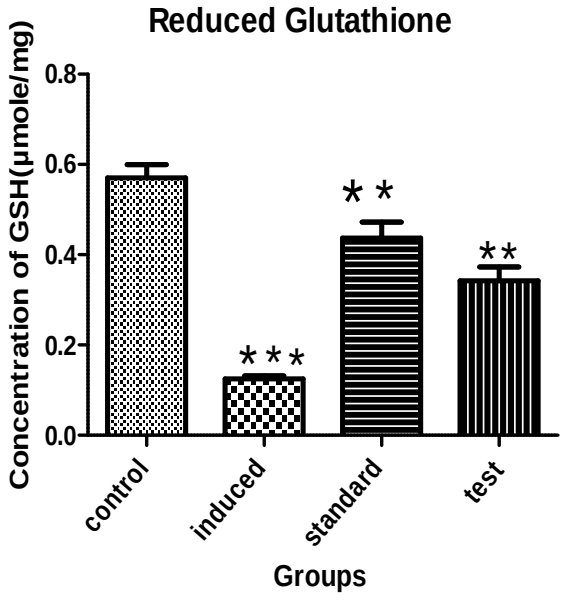


Figure 8: Estimation of reduced glutathione

Discussion:

Neuropathic pain effects millions of people worldwide causing substantial disability and greatly impairing quality of life. Commonly used analgesics and antihyperalgesic compounds are generally characterized their limited therapeutic outcome, thus there is a compelling need for novel therapeutic statergies able to prevent nervous tissue alteration responsible for chronic pain.

The seeds of Cucurbita pepo was extracted and isolated to obtain phenolic compounds and Vanillin was the main target for study. Phytochemical screening of isolated extract showed the presence of various polyphenols such as phenol, flavonoids, alkaloids, saponins, and carbohydrates. Thin layer chromatography showed the presence of Vanillin in extract. The isolated compound was subjected to determine the preliminary toxicological studies, and it was found safe at highest dose of 2000mg/kg. Invitro antioxidant activity and invivo anti neuropathic pain activity was carried out in animal models at the dose level of 400mg/kg which is $1/5^{th}$ of the human dose.

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- Diphenyl-2-Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured at an absorbance of 517 nm. Thus it was found that Phenolic extract of *C.pepo* seeds showed a good antioxidant activity when compared with standard quercetin.

Pyridoxine (vitamin B6) intoxicated rats develop a peripheral neuropathy characterized by sensory nerve conduction deficits associated with disturbances of nerve fiber geometry and axonal atrophy. The rationale for selecting pyridoxine to produce an animal model of large-fiber neuropathy is based on several factors, including the selective and severe neurotoxic actions of this compound on large DRG neurons in rodents, dogs, and humans. Present study of animal model of sensory neuropathy is that the rapidly developing, large fiber neurodegeneration may be considered to model one aspect of clinical diabetic peripheral neuropathy. This model could be of use as a screen for evaluating neurotrophic / neuroprotective properties of novel compounds currently in development for type 2 diabetes mellitus.

Results of the present study indicated that Phenolic extract of *C.pepo* seeds(400 mg/kg) is safe for the nervous system and could help to improve the function of the nerve Fibers. Inter group comparison by one way anova in tail flick latency method on day 7 and

day 14 ($P<0.0001$) showed significant decrease in tail flick latency in comparison to day 0, and also test group and positive control group showed significant decrease in tail flick latency when compared with pyridoxine control group.

Alcoholic Neuropathy was induced by administration of 10g/kg of 35% v/v ethanol twice a day. The development of neuropathic pain following chronic ethanol consumption is well reported. Alcoholic Neuropathy was developed in a total period of seven weeks and biochemical parameters were assessed on the last day. The concentration of alcohol was selected on the basis of previously reported studies. Phenolic extract of *C.pepo* seeds(400 mg/kg) was given one hour before ethanol administration. Pregabalin (2 mg/kg i.v) was used as a positive control.

In the present study, the chronic administration of ethanol was resulted in marked reduction in thermal hyperalgesia and mechanical allodynia. These results were reliable when compared with the previous reports demonstrating neuropathic pain like state in the rats following chronic alcohol consumption.

According to some studies, *C.pepo* seeds exhibits an analgesic and antioxidant effect by bringing about a reduction in pain, inflammation and the signal transduction pathway which results in a decline in plasticity at dorsal root of spinal cord through deprivation in P substance. Therefore, in this study, the continuous administration of phenolic extract of *C.pepo* decreased pain perception and treated thermal hyperalgesia and mechanical allodynia.

Oxidative injury has been implicated in pathophysiology of alcoholic neuropathy. ROS triggers second messengers which are involved in central sensitization of dorsal horn cells. It also activates spinal glial cells which play an important role in chronic pain. Nitric oxide is also implicated in neuropathic pain. It sensitizes spinal neurons and also contributes to the sensitization of central neurons by disinhibition. Reduced glutathione is a major low molecular weight scavenger of free radicals in cytoplasm. Depletion of glutathione increases the susceptibility of neurons to oxidative stress and hyperalgesia . A significant increase in TBAR levels, and reduced glutathione were observed in the sciatic nerve of ethanol treated rats.

The administration of Phenolic extract of *C.pepo* also exhibited an antioxidant effect due to presence of phenolic compounds. The mechanisms by which Phenolic extract of *C.pepo*

brings about its antioxidant effect is by free radical scavenging, chelation of transition metal ions, and inhibition of oxidases. It also inhibits lipid peroxidation. Henceforth, treatment with Phenolic extract of *C.pepo* produced significant protection in alcoholic neuropathy as evident from improvement in the reduction in nociception.

On the basis of above, it may be concluded that abrogation in Alcoholic Neuropathy with repeated oral administration of extract may alleviate established behavioral and biochemical symptoms of neuropathic pain, possibly through analgesic, antioxidant effect, reduction in TBARS and increase in glutathione level in alcohol induced neuropathic pain.

CONCLUSION:

In conclusion the findings represented that Phenolic extract of C.pepo with vanillin as a focused compound showed a good analgesic and antioxidant activity and posses an ameliorative effect in neuropathic pain induced models.

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