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Original Research Article

Evaluation of analgesic activity of sodium valproate and ethanolic extract of *Vitex negundo* in experimental analgesic models in wistar rats

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ABSTRACT

Background: NSAIDs and opioids are commonly prescribed medications to relieve pain of multiple aetiologies with no effect on the level of consciousness of the patient. They interfere with the mode of transmission of the pain message. A widely prescribed antiepileptic drug, sodium valproate has been used in various non-epileptic conditions like migraine prophylaxis and in the treatment of bipolar disorder because of the multiple mechanisms by which it acts. *Vitex negundo* has been investigated for antipyretic, analgesic, anti-inflammatory, anticonvulsant, hepatoprotective and bronchial relaxant. Very few studies have been done to evaluate its analgesic activity and no study was done on analgesic activity with the combination of modern drug. The more important point to be noted is that *Vitex negundo* is a natural product and therefore unlikely to cause adverse effects when compared to the traditional drugs used to treat pain. The aim of the present study was to evaluate of analgesic activity of sodium valproate and docosahexaenoic in experimental analgesic models in wistar rats.
Methods: For analgesic activity, a total of 36 adult Wistar albino rats were taken and divided into six groups of six rats each. Group I was control (distil water 1ml/kg), Group II received intraperitoneal injection of diclofenac sodium (10mg/kg), Group III, IV were injected intraperitoneal sodium valproate 200, 400mg/kg with distil water respectively and Group V, VI were given sodium valproate 200, 400mg/kg (intraperitoneal) plus EEVN 400mg/kg (orally) respectively. Analgesic activity was assessed using hot plate, tail flick and acetic acid writhing models.
Results: Present study revealed that sodium valproate at higher doses (400mg/kg) used either alone along with EEVN (400mg/kg) showed statistically significant analgesic activity in comparison to control in various experimental models for assessing pain.
Conclusions: Combination of sodium valproate along with EEVN has shown promising analgesic activity.

Keywords: Analgesia, Antiepileptic, Acetic acid writhing, Hot plate, Tail flick

INTRODUCTION

Pain has been defined by the international association for the study of pain (1979) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage."[1]

Pain is an unpleasant sensation localized to a part of body. It is often described in terms of a penetrating or tissue-destructive process (twisting, stabbing tearing, squeezing and burning) and/or of a bodily or emotional reaction (terrifying, nauseating, and sickening). Furthermore, any pain of moderate or high intensity is accompanied by

anxiety and an urge to escape or terminate the feeling. These symptoms depict the dual nature of pain: it is both sensation and emotion. When acute, pain is characteristically associated with behavioural arousal and a stress response consisting of increased blood pressure, heart rate, pupil diameter and plasma cortisol levels.[2] Pain is a pervasive public health problem, and analgesic drugs play a central role in its treatment.[3] Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are currently the most widely prescribed drugs for the treatment of pain and inflammation. They are more effective in controlling painful inflammatory conditions like osteoathritis, ankylosing spondylitis, rheumatoid arthritis etc. However, there are number of side effects associated with the use of NSAIDs. The traditional NSAIDs usually cause gastrointestinal adverse effects on long term use and the selective cyclooxygenase (COX2) inhibitors are known to possess dangerous cardio toxic potential.[4] The other group of drugs used in the management of pain is opioid analgesics which are having the good pain-relieving activity. However, in patients with chronic pain opioids are rather avoided due to its addiction liability and abuse potential.

Researchers have been continuously exploring newer molecules to develop an ideal analgesic which is non-toxic as well as effective at the same time. A widely prescribed antiepileptic drug, sodium valproate has been used in various non-epileptic conditions like migraine prophylaxis and in the treatment of bipolar disorder because of the multiple mechanisms by which it acts.[5] Sodium valproate is an inhibitor of Histone Deacetylation (HDAC). Rat stroke models in the past have demonstrated the neuro-protective effect of valproate due to HDAC inhibition.[6]

Vitex negundo Linn (Family: Verbenaceae) is a woody, aromatic shrub growing to a small tree. Some common names are in Hindi nirgundi and in Sanskrit as sindhuvara. All parts of the plant from root to fruit possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. *Vitex negundo* has been investigated for antipyretic, analgesic, anti-inflammatory, anticonvulsant, hepatoprotective and bronchial relaxant.[7-11] Very few studies have been done to evaluate its analgesic activity and no study was done on analgesic activity with the combination of modern drug. The more important point to be noted is that *Vitex negundo* is a natural product and therefore unlikely to cause adverse effects when compared to the traditional drugs used to treat pain.[12]

From the view of various existing reports, authors initiated a study to evaluate the analgesic activity of sodium valproate and ethanolic extract of *Vitex negundo* in experimental analgesic models in wistar rats.

METHODS

An in vivo experimental study conducted after taking the approval from the institutional animal ethics committee.

Chemicals

Three drugs were used during the study; diclofenac sodium (Cipla), sodium valproate (Zydus Pharmaceuticals) and ethanolic extract of *Vitex negundo* (EEVN) were used. The pure powder form of sodium valproate was used, dissolved in distilled water. Acetic acid used for writhing test.

Equipment's

Feeding tube, Eddy's hot plate, tail flick analgesiometer were used, Soxhlet apparatus.

Plant preparation and extraction

The fresh leaves were purchased from the local market and the leaves of *Vitex negundo* were shade dried and coarsely powdered. The powder is filled in to filter paper bag and placed in the soxhlet apparatus for extraction. The soxhlet apparatus is connected to round bottom flask which is fill by ethanol (90%) solvent and water bath to maintain temperature. The ethanol was boiled at 40°C for over a period of 24hours. The extract obtained was 20% and was stored in desiccator at room temperature

Selection of the animal

Thirty-six male albino Wistar healthy rats were taken from the central animal house for the study. The animals weighed 150g to 200g and the animals were housed under standard condition, housed individually with normal water and food granules, 12:12 hours light dark cycle, 50% humidity and 28°C temperature and provided with standard food granules and water ad libitum. These animals were individually housed in poly propylene cages which contained paddy husk (procured locally) which was sterile.

Identification of individual animals

Rats from each treatment group were marked using different coloured marks on their tails, for easy identification and experimentation, according to the group allotted.

Sample size

The minimum number of rats in each group was kept as six, so as to obtain statistically significant results within and between the groups.

Evaluation of analgesic activity

A total of 36 adult Wistar albino rats were divided into six groups. Each group contained six rats. They were weighed and marked. Group I was control (distilled water 1ml/kg), Group II received intraperitoneal injection of diclofenac sodium (10mg/kg), Group III, IV were injected intraperitoneal sodium valproate 200, 400mg/kg dissolved with distilled water and Group VI, VII, were given sodium

valproate 200, 400mg/kg dissolved with distilled water along with EEVN 400mg/kg (orally) respectively. The dose of sodium valproate was chosen as 200mg/kg and 400mg/kg based on a study performed by Raza M et al.[13] The dose of EEVN was chosen as 400mg/kg based on a study done by Maniyar YA et al.[14]

Table: 1 Animals were divided in to 5 treatment groups 6 rats in each group.

Group	No. of animals
Group 1 Control - Distilled water	6
Group 2 Standard-Diclofenac Sodium	6
Group 3 Sodium Valproate 200mg/kg	6
Group 4 Sodium Valproate 400mg/kg	6
Group 5 Sodium Valproate 200mg/kg + EEVN 400mg/kg	6
Group 6 Sodium Valproate 400mg/kg + EEVN 400mg/kg	6

Eddy's hot plate method

The albino wistar rats were weighed and marked. Each rat was placed on the Eddy's hot plate. Temperature was maintained at constant temperature of 55-56°C. The time taken by the animal for either licking the paw or withdrawal of the paws or jumping off the surface, whichever observed first, was taken as the end point. Reaction time was measured by stopwatch in 1/100 s increments and was taken before baseline and 30, 60 and 120 min after the drug or vehicle administration in each animal. An increase in the time interval of withdrawal latency was indicative of analgesic activity. In order to avoid injury to the tissue a cut off of 60 seconds was set.

Tail flick method

Analgesia was measured using tail flick method also called the modified D Amour and Smith method.[15] The albino Wistar rats were weighed and marked. The response was noted by measuring the latency of the response (tail flick) with the help of tail flick test. The Wistar rats were placed in the restrainer and their tails were placed on an analgesiometer. The baseline responses were recorded just before administering the drug followed by 15th, 30th, 60th, 90th and 120th minute post administration of the drug by giving latent heat using a heated nichrome wire at 1cm - 1.5cm from the tail tip at the dorsal surface. Withdrawal latency is then recorded as the time between placing the rat tail on the heat source till the sudden sharp tail withdrawal. In order to protect tail from thermal injury, a cut off time is set at 10 seconds while checking the tail flick reaction time.[16]

Writhing method

This test is a type of chemical pain /nociceptive test and the main mechanism for pain production is based on irritant induced peritonitis. 12hours prior to the test, food was withdrawn, and no food was given till the completion of the test.

The albino Wistar rats were weighed and marked. The test drugs were injected according to the group and after 30minutes of post administration of test drug, 2% acetic acid was injected intraperitoneally in order to produce writhing. Ten minutes post administration of 2% acetic acid writhing movements like elongation of body, abdominal constriction, trunk twisting, forelimb extension, hind limb extension and back arching were counted cumulatively for a period of 20minutes.

Statistical analysis

The results will be analysed using one way ANOVA followed by post-hoc Tukey's test in SPSS 21 Software for Microsoft. The statistically significant value for any measure was set to $p < 0.05$ at a confidence interval of 95%. The results expressed is in mean±standard deviation

RESULTS

Hot plate method

The pain reaction was significantly increased with the standard group diclofenac sodium 10mg/kg to 11.34±0.38, 13.44±0.32 and 16.02±0.41 at 60, 90 and 120th minute respectively when compared to control group 4.8±0.36. The pain reaction in group 4 sodium valproate 400mg/kg was also significantly increased to 10.48±0.22, 12.09±0.60, 14.00±0.18 at 60, 90 and 120th minute respectively when compared to control group 4.8±0.36.

There is also significance increase in reaction time seen with group 5 the sodium valproate 200mg/kg plus EEVN 400 mg/kg when compared to control group 11.06±0.41, 11.48±0.22, 12.08±0.33 at 60, 90, 120th minute. Group 6 sodium valproate 400mg/kg plus EEVN 400mg/kg shown high significance with control group when compared to other groups 12.08±0.44, 13.58±0.62, and 14.28±0.52 at 60, 90 and 120th minute (Table 2).

Tail flick method

The tail flick time or withdrawal response was significantly increased to 8.7±0.30 in the diclofenac sodium 10mg/kg treated group, 7.56±0.40 in the sodium valproate 400mg/kg group and 8.16±0.35 in the sodium valproate 400mg/kg plus EEVN 400mg/kg group at 90 minutes when compared to control.

The tail flick time or withdrawal response was significantly increased to 9.35±0.18 ($p < 0.001$) in the diclofenac sodium 10 mg/kg treated group, 8.58±0.42 in the sodium valproate 400mg/kg group and 9.37±0.23 in the sodium valproate 400mg/kg plus EEVN 400mg/kg at 120 minutes when compared to control 5.15±0.31 (Table 3).

Table 2: Eddy's hot plate method - retention time at different time points (in seconds).

Groups	0 min	15 min	30 min	60 min	90 min	120 min
Control	3.52±0.21	3.51±0.26	4.8±0.36	4.19±0.17	3.27±0.39	3.48±0.21
Standard -diclofenac sodium	6.35±0.59	6.93±0.58[a]	9.28±0.29[a]	11.34±0.38[ab]	13.44±0.32[ab]	16.02±0.4[ab]
Sodium valproate 200mg/kg	5.72±0.86	5.98±0.44	6.01±0.28	6.18±0.22[c]	6.24±0.73[c]	6.44±0.39 [c]
Sodium valproate 400mg/kg	5.88±1.38	6.43±2.86	7.04±0.56	10.48±0.22 [ab]	12.09±0.60 [ab]	14.00±0.18[ab]
Sodium valproate 200mg/kg EEVN 400mg/kg	4.13±0.19	6.28±1.16	7.33±1.02	11.06±0.41[ab]	11.48±0.22 [ab]	12.08±0.33[b]
Sodium valproate 400mg/kg + EEVN 400mg/kg	6.04±0.48	7.08±0.22	7.48±0.33	12.08±0.44[b]	13.58±0.62[ab]	14.28±0.52[ab]

All values are expressed as Mean±SEM; a denotes p <0.05 compared to baseline; b denotes p <0.05 compared to control; and c denotes p <0.05 compared to standard; p-value obtained by one-way ANOVA followed by Post-Hoc Tukeys test.

Table 3: Tail flick method-retention time at different time points (in seconds).

Groups	0 min	15 min	30 min	60 min	90 min	120 min
Control	4.58±0.2	5.08±0.23	5.27±0.12	5.20±0.48	5.28±0.21	5.15±0.31
Standard - diclofenac Sodium	3.58±0.14	4.47±0.22	5.54±0.32[a]	6.58±0.18[a]	8.7±0.3[ab]	9.35±0.18[ab]
Sodium valproate 200mg/kg	5.62±0.76	5.88±0.24	6.21±0.28	6.30±0.12	6.34±0.53[c]	6.54±0.38[c]
Sodium valproate 400mg/kg	6.08±1.88	6.33±2.86	7.24±0.56	10.18±0.12	7.56±0.40[b]	8.58±0.42[ab]
Sodium valproate 200mg/kg + EEVN 400mg/kg	4.23±0.19	6.48±1.16	7.43±1.02	10.06±0.41	11.48±0.22[c]	12.08±0.33[c]
Sodium valproate 400mg/kg + EEVN 400mg/kg	6.44±0.38	7.28±0.21	7.58±0.33	12.58±0.24[a]	8.16±0.35[ab]	9.37±0.23[ab]

All values are expressed as Mean ±SEM; a denotes p <0.05 compared to baseline; b denotes p <0.05 compared to control; and c denotes p <0.05 compared to standard; p-value obtained by one-way ANOVA followed by Post-Hoc Tukeys test.

Writhing test method

The number of abdominal constrictions or writhing were significantly reduced to 5.48±0.58 in the diclofenac sodium 10mg/kg treated group, 21.63±2.81 in the sodium valproate 200mg/kg group, 7.58±1.66 in the sodium valproate 400mg/kg group, 18.14±1.02 in the sodium valproate 200mg/kg plus EEVN 400mg/kg group and 6.07±1.28 in the sodium valproate 400mg/kg plus EEVN 400mg/kg group when compared to control 36.5±4.8 (Table 4).

Table 4: Writhing test response.

Groups	Writhing response
Control	34.5±4.4
Standard - Diclofenac Sodium	5.48±0.58[b]
Sodium Valproate 200mg/kg	21.63±2.81[bc]
Sodium Valproate 400mg/kg	7.58±1.66[b]
Sodium Valproate 200mg/kg + EEVN 400mg/kg	18.14±1.02[bc]
Sodium Valproate 400mg/kg + EEVN 400mg/kg	6.57±1.28[b]

All values are expressed as Mean±SEM; b denotes p <0.05 compared to control; and c denotes p<0.05 compared to standard p-value obtained by one-way ANOVA followed by Post-Hoc Tukey's test.

DISCUSSION

NSAIDs and opioids are commonly prescribed medications to relieve pain of multiple aetiologies with no effect on the level of consciousness of the patient. They interfere with the mode of transmission of the pain message.

The present study was carried out using different experimental pain models to evaluate analgesic activity of sodium valproate and EEVN. The analgesic models in our experiment was checked using Eddy's hot plate, tail flick and acetic acid writhing models which are standard models for screening agents for analgesic activity.

In the hot plate model sodium valproate 400mg/kg, sodium valproate 200mg with 400mg of EEVN and sodium valproate 400mg with 400mg EEVN showed significant analgesic activity compared to control group at 60 minutes, 90minutes and 120minutes. In the tail flick model, sodium valproate given at 400mg and sodium valproate 400mg with 400mg of EEVN showed significant analgesic activity when compared to control at 90 and 120minutes. In the acetic acid Writhing model, sodium valproate given at a dose of 200mg, sodium valproate given at a dose of 400mg, sodium valproate 200mg with 400mg of EEVN and sodium valproate 400mg with 400mg of EEVN showed significant analgesic activity as compared to control. It has been reported that sodium valproate possessed analgesic activity

when used alone.[17] Similarly Nakamoto K et al, reported that EEVN when used alone possessed analgesic activity Manirujjaman et al, but the co-administration of EEVN along with sodium valproate has potentiated the analgesic activity of the later.[18]

The analgesic activity of sodium valproate can be attributed to the increase in glutamate transporters like glial Glutamate Transporter 1 (GLT 1) and Glutamate Aspartate Transporter (GLAST), in dorsal horn of the spinal cord and also in the hippocampus. This action of sodium valproate is due to inhibition of histone deacetylation.[19] Mesdjian et al, attributes the analgesic activity of sodium valproate to its effect on GABA neurotransmission. Sodium valproate is said to increase the GABA neurotransmission causing analgesia.[20]

Similar to sodium valproate, Similarly *Vitex negundo* possess analgesic activity via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key mediators in pain. Previous studies suggested that alkaloids also involve in analgesic action through non-narcotic action.[21-24] In the present study, flavonoids, tannins and alkaloids might be attributed to the peripheral and central analgesic activities. This may be the probable reason for potentiating the analgesic activity of sodium valproate by EEVN.

Limitations of this study were a single dose of EEVN has been selected in last two groups. The dose dependent effects were not known. Mechanisms mentioned in previous studies were considered instead. The mechanisms by which sodium valproate and EEVN showed analgesic activity was not studied.

CONCLUSION

In conclusion, the combination of sodium valproate and EEVN has shown promising analgesic activity and was almost comparable to standard drugs used in this study. However, no clear inference can be drawn at this stage and hence we consider the work for further extensive research.

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