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A Novel Gabapentin Analogue Assuages Neuropathic Pain Response in Chronic Sciatic Nerve Constriction Model in Rats

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1. Introduction

Injury to the peripheral nervous system can lead to abnormal pain conditions collectively referred to as neuropathic pain [1]. It includes a large number of heterogeneous pain syndromes with diverse etiologies, which however, typically exhibit frequent disordered somatosensory attributes such as hyperalgesia, allodynia, paroxysmal spontaneous pain, and paraesthesia [2]. It is therefore likely that some of the inherent mechanisms of neuropathic pain may be shared by different syndromes [3]. This pain syndrome comprises of some particular somatosensory disorders. The most salient symptoms include allodynia and hyperalgesia. In reality, allodynia and hyperalgesia can be elicited by both mechanical and thermal (hot/cold) stimuli [4, 5]. The condition may also arise following other pathological states such as diabetes mellitus, HIV infection, mechanical trauma, tumor invasion, metabolic diseases, neurotoxic chemicals, infections, and autoimmune diseases. It involves multiple pathophysiological changes in autonomic and central nervous systems [6-8] and rarely responds to conventional analgesics [9-12]. The management of patients with chronic neuropathic pain is therefore not straightforward and the effectiveness of current treatments is unpredictable with a delayed onset of analgesia and a high side effect profile [13].

Currently, the pharmacological management of neuropathic pain is based chiefly on the utilization of anticonvulsant and antidepressant drugs rather than opioids whose use is considered controversial [14]. Moreover, drugs which are marketed for the management of neuropathic pain are less than optimal and mitigate pain in only about 50% of patients, so therapy of neuropathic pain is a notable unmet medical challenge [13, 15]. At present, in the anticonvulsant drug category, GBP and pregabalin (PGB), are the most frequently prescribed [8, 16, 17] while the tricyclics are the those of choice among the antidepressants [14, 18-20].

The paucity of efficacious low side effect drugs for neuropathy has stimulated the quest for a new drug capable of minimizing neuropathic pain. The Chronic constriction injury model (CCI model) is considered as an exclusive model of post-traumatic painful peripheral neuropathy [21]. We have therefore developed a derivative incorporating salicylaldehyde as a substituent on the amine functionality of the GBP compound [Gabapentsal (GPS)], (Fig 1B) by a novel method and then studied its effectiveness, previously in cisplatin induced neuropathy [22], and now in the CCI model of neuropathy in rats in order to further validate the therapeutic potential of this compound. It has been recommended that conjugation of mechanistically distinctive analgesic agents may come up with additive results or synergism at smaller doses, with minimal side effects than either of drugs when used separately [23-26]. In this setting, a rationale was adopted using the GBP (Fig 1A) derivative based on the assumption that combining the reported antineuropathic effects of GBP [27, 28] with the antinociceptive and anti-inflammatory properties of salicylaldehyde [29] in a single molecule would display potential benefit over GBP.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats, weighing 250–350 g and kept in a 12 h-12 h light-dark cycle at 22.0 ± 2.0° C were utilized in the study with food and water available *ad libitum*. All the experimental procedures on animal subjects were executed in harmony with the UK Animals (Scientific Procedures) Act 1986, ARRIVE guidelines and in conformance with the regulations outlined by the committee on animal research ethics, Department of Pharmacy, University of Peshawar. Endorsement for the study was granted under the registration # 10/ EC-15/Pharm. To eliminate any possible bias, all animal groups were coded and an experienced investigator was blinded to the treatments.

2.2. Induction of neuropathic pain

Chronic constriction injury (CCI) model was used to induce neuropathic pain (Bennett and Xie 1988). In brief, each animal was anaesthetized with an intraperitoneal (i.p) injection of xylazine (10 mg/kg) and then placed in a prostrate position on a heat-regulated pad. Lubricating ophthalmic ointment (Lacri-Lube®; Barrett Hodgson, Karachi, Pakistan) was applied to the eyes to avoid dryness.

The left thigh was elevated and the pelt on the posterior skin was shaved. The exposed skin was swabbed with a povidone iodine topical 10% w/v. A 3–4 cm incision was made parallel to the long axis of the femur and down the center of the biceps femoris muscle. Under a magnifying glass, the connective tissue between the gluteus superficialis and the biceps femoris muscles was cut. Using ribbon retractors, the gap between the two muscles was widened and approximately 10 mm of the sciatic nerve (proximal to the sciatic trifurcation) was freed from the surrounding connective tissue with a micro-scissor. Four ligatures (Chromic catgut suture 4/0, metric 2; Ethicon, Karachi, Pakistan), each with a double knot, were tied 1.0 mm away from each other and proximal to the trifurcation of the sciatic nerve.

Constriction of the nerve was kept minimal and straight away discontinued when a succinct twitch was observed. The muscle layer was then sutured (Silk braided 2/0, metric 2; Zhejiang Medicines and Health Products, Hangzhou, Zhejiang, China) and the skin was closed using a surgical stapler (Surgical stapler (Manipler AZ-35W, B. Braun Surgical S A, Rubi, Spain). In sham-operated rats, an identical procedure was conducted, without sciatic nerve ligation [30].

2.3. Treatment groups

Animals were randomized into different treatment groups (n=6, per group). Baseline allodynia and hyperalgesia assessments in normal animals were taken 5 days before surgery. Then the first reading following CCI was carried out on the protocol 7th experimental day. On day15, the pre-and post-dose (1 and 3 h) assessments were subsequently performed (Fig. 2).

2.4. Static mechano-allodynia

A sequence of 8 von Frey hairs (0.6,0.4,1,2,4,6,8,15 g force) (Stoelting, Wood Dale, Illinois, USA) was applied perpendicularly to the mid-plantar surface of the operated left hindpaw to an degree that caused the hairs to buckle [31]. Each von Frey filament was applied for up to 6 s as a cut-off time or till a positive reaction was observed. Paw withdrawal or flinching immediately after application of the filament was registered as a positive response and a next von Frey filament of smaller force was applied for the ensuing assessment.

In case of the lack of any response, the subsequent von Frey hair of stronger force was employed. This practice was continued until 4 measurements were recorded after an initial change in direction (+ ve response) or five consecutive negative responses (2, 4, 6, 8, 15 g force) or four repeated positive responses (2, 1, 0.6, 0.4 g force). A force of 15 g was chosen as the cut-off at which point additional application was concluded. von Frey filaments were applied at a gap of several seconds to avert any impact of earlier stimuli on animal behavior. Any ambulation was noted as an indistinct response, and the stimulus was applied again. The pattern (xo) of response was converted to 50% paw withdrawal threshold (PWT, g) as described by Mao-Ying and coworkers [32, 33] based on a previously described method [31].

2.5. Dynamic mechano-allodynia

Dynamic mechano-allodynia was evaluated by gently stroking the mid-plantar surface of the operated left rear paw with a cotton bud. Flinching or licking of the paw was recognized as a withdrawal response and the time elapsed till the onset was recorded as the paw withdrawal latency (PWL). A period of 15 s was inflicted as the cut-off time [34].

2.6. Heat hyperalgesia

The operated left hind paw was lightly touched at the mid region of the plantar surface with a heated plate maintained at a constant temperature (56.0 °C). The heat source was applied to evoke a paw flick response and both the paw withdrawal latency (PWL) and duration (PWD) were then measured up to a maximum cut-off latency of 10 s [21].

2.7. Mechano-hyperalgesia

Animals were placed on an elevated framework and the tip of an ordinary office paper pin was pressed against the skin of the mid-plantar surface of the operated left hind-paw such that the skin was dimpled but not pierced (prick test). The latency to paw withdrawal (PWD) was documented, with a discretionary nominal time of 0.5 s (for the short normal response) and a maximal cut-off of 15.0 s [35].

2.8. Cold allodynia

A 50 uL volume of acetone was carefully sprayed onto the mid-plantar surface of the operated left hind paw without touching the skin, using a blunt needle coupled to a syringe plunger. The duration of the withdrawal response (PWD) was measured with a discretionary minimal value of 0.5 s and a maximum of 15.0 s [35].

2.9. Locomotor activity

General locomotor activity was appraised in an open field setting consisting of a behavioral arena with a floor area of 50 × 40 cm distributed into four equal quadrants by lines [36]. On the test day, each rat was habituated to the test apparatus for 30 min. The incidence of line crossing by each animal was counted for 20 min, 1 and 3 h post systemically administered GBP, test drug or vehicle treatment. Locomotor activity was subsequently recorded post surgically on days 7 and 15.

2.10. Motor coordination

2.10.1. Rota rod

A purpose built accelerating rotarod was used whereby animals were mounted on a rotating drum with increasing velocity from 4 to 40 rpm over 5 min. This impelled animals to walk forward in order to avoid falling off [37, 38]. The latency to rotarod dismount was recorded and each animal was given three trials on the test day before drug treatment. Motor performance was evaluated on day -5 (pre-trial) and after surgical intervention on days 7 and 15 with motor function assessment at 1 and 3 h post systemic administration of GBP/GPS/vehicle.

2.11. Chemicals

GBP was obtained from Lowitt Pharmaceuticals, Peshawar Pakistan. GPS was synthesized as described [22], Xylazine (Xylaz, 20 mg/mL, Farvet Laboratories, Bladel, Netherlands) and ketamine (Ketarol, 50 mg/mL; Global Pharmaceutical, Islamabad, Pakistan). Povidone iodine topical 10 % w/v solution (Pyodine; Brookes Pharma, Karachi, Pakistan), Lubricating ophthalmic ointment (Lacri-Lube; Barrett Hodgson, Karachi, Pakistan).

2.12. Statistical analysis

Results are presented as mean ± SD. Multiple group means of parametric data sets were compared using two-way analysis of variance (ANOVA) after it was determined that the data conformed to a normal distribution with equal variances. If an overall significance was found, Bonferroni's multiple-comparison test was employed using GraphPad Prism 5 (GraphPad Software Inc. San Diego CA, USA). A value of $P < 0.05$ was admitted as significant.

3. Results

3.1. General behavior of animals

No changes in general behavior and social interaction were observed between the CCI animals and the naive and sham operated controls. Autotomy was observed in about 1 % of all the ligated animals and these were excluded from the study and immediately euthanized. A comparable increase in body weight was observed in all experimental groups.

3.2. Activity of GBP and GPS on CCI induced static mechanical allodynia

Animals subjected to the CCI procedure expressed rapid responses to the von Frey filament stimulus in contrast to sham operated animals (day 7-15, $P < 0.001$, Fig. 3). The paw withdrawal threshold decreased from a pre-CCI value of 15.0 g to a protocol day 15 post-CCI value of 0.9 g. Statistical analysis revealed a significant main dose effect on CCI induced mechanical allodynia [time = (F (4, 175) = 372.0, $P < 0.0001$), treatment = (F (6, 175) = 132.9, $P < 0.0001$), interaction = (F (24, 175) = 15.6, $P < 0.0001$)]. CCI-GBP (100 mg/kg) treated animals displayed resistance to the von Frey filament pressure at1 and 3 h test intervals (7.4 ± 2.5 g and 9.2 ± 3.9 g, $P < 0.005$) respectively compared to pre GBP administration (7.0 ± 6.2 g, $P > 0.05$) on day 15. Drug naive CCI-animals exhibited a persistent allodynic response from day 7-15 [(day 7: 1.8 ± 1.1 g , $P < 0.001$), (day 15, pretreatment: 0.9 ± 0.7 g, $P < 0.001$), (day 15,1 h post treatment: 0.9 ± 0.7 g)

and (day 15,3 h post treatment: 0.8 ± 0.8 g)] within the experimental protocol when compared to the sham operated animals [(day 7: 14.1 ± 1.6 g, P <0.001), (day 15 pretreatment: 13.7 ± 1.1 g, P <0.001), (day 15, 1 h post treatment: 14.0 ± 1.9 g, P <0.001) and day (day 15, 3 h post treatment: 14.4 ± 1.8 g, P <0.001)]. Animals treated with GPS significantly increased the CCI induced paw withdrawal threshold to gentle static von Frey filament pressure when tested at 1 and 3 h post-doses of 25 mg/kg (3.5 ± 1.3 g and 3.5 ± 0.9 g, P >0.05), 50 mg/kg (4.2 ± 1.7 g and 5.3 ± 1.1 g, P <0.01, P <0.001), 75 mg/kg (5.5 ± 1.9 g and 7.4 ± 3.3 g, P <0.001) and 100 mg/kg (9.5 ± 1.6 g and 11.3 ± 2.8 g, P <0.001) on day 15 of the experimental protocol.

3.3. Activity of GBP and GPS on CCI induced dynamic mechano-allodynia

Dynamic mechano-allodynia was manifested by animals subjected to CCI (P <0.001) versus sham operated controls when tested on the 15th protocol day (Fig.4). In contrast, an anti-allodynic response was noted after treatment with either GBP or GPS [time = (F (4, 175) = 344.7, P <0.0001), treatment = (F (6, 175) = 64.4, P <0.0001), interaction = (F (24, 175) = 15.0, P <0.0001). In the case of GBP, the anti-allodynic response values were 5.5 ± 1.0 s and 6.0 ± 2.2 s (P <0.01) compared to CCI-vehicle treated control values of 2.8± 1.5 s and 3.2 ± 1.2 sat 1 and 3 h post-administration respectively. Whilst the values for GPS treated animals were dose-related at doses of 50 mg/kg (7.0 ± 1.8 s and 8.2 ± 2.1s, P <0.001), 75 mg/kg (9.0 ± 1.9 sand 10.7 ± 1.6 s, P <0.001) and 100 mg/kg (11.2 ± 2.3 s and 12.5 ± 1.0 s, P <0.001) at 1 and 3 h post-administration respectively. GPS (25 mg/kg) appeared to be a sub-threshold dose because it failed to attenuate the CCI-induced enhanced sensitivity to the cotton bud dynamic allodynia stimulus (3.3 ± 1.9 s and 4.2 ± 1.9 s, P >0.05 respectively 1 and 3 h post drug administration). Sham-operated animals on the other hand displayed a higher paw withdrawal latency to the stimulus (13.3 ± 2.3 s and 14.2 ± 1.3 s, P <0.001) when compared to the CCI-vehicle treated group (2.8 ± 1.5s and 3.2 ± 1.2s).

3.4. Activity of GBP and GPS on CCI induced thermal hyperalgesia (PWD)

In the CCI-rat model, GBP induced an inhibition of thermal hyperalgesia increasing a pre-CCI paw withdrawal latency of 0.5 s (-5 day) up to a 14 s duration on the15th day post-CCI (Fig. 5). An overall main dose effect was observed after treatment with either GBP or GPS [time = (F (4, 175) = 480.6, P <0.0001), treatment = (F (6, 175) = 176.7, P <0.0001), interaction = (F (24, 175) = 20.83, P <0.0001). Thus, GBP (100 mg/kg) administered to the CCI group displayed a decrease (8.3 ± 2.2 s and 6.5 ± 1.6 s, P <0.001) in the paw withdrawal duration compared to the CCI-vehicle controls (14.0 ± 0.9 s, P < 0.001) on day 15 at 1 and 3 h post drug administration.

On protocol day 15, compared to the CCI-vehicle treated animals (PWD = 14.0 ± 0.9 s, P <0.001), GPS evoked a dose dependent decrease in withdrawal duration over the range starting from 25 (11.8 ± 1.5s, P <0.01 and 10.7 ± 1.4s, P <0.001), 50 (8.2 ± 1.7 s and 7.7 ± 1.4 s, P <0.001), 75 (6.8 ± 1.7 s and 5.8 ± 1.2s, P <0.001) and 100 mg/kg (5.3 ± 1.6 s and 5.0 ± 1.1 s, P <0.001) at 1and 3 h post administration.

3.5. Activity of GBP and GPS on CCI induced heat hyperalgesia (PWL)

GBP (100 mg/kg) induced a rise in paw withdrawal latency (8.0 ± 1.4 s, P <0.001) and (9.8 ± 1.8 s, P <0.001) at 1 and 3 h, respectively, post treatment on the15th CCI protocol day compared to vehicle treated controls (1.7 ± 0.8s, P <0.001) (Fig. 6). ANOVA analysis of left paw reaction latencies indicated a main group effect of both GBP and GPS [time = (F (4, 300) = 413.1, P < 0.0001), treatment = (F (11, 300) = 148.4, P <0.0001), interaction = (F (44, 300) = 19.4, P <0.0001). A graded dose response relationship was observed with GPS treatment (25-100 mg/kg). *Post hoc* analysis revealed that all CCI-GPS treated groups showed a significant increase in PWL compared to the vehicle control at 1and 3 h post administration on day 15 of the CCI procedure. Injection of GPS generated discernable effects after 1 and 3 h at doses of 25 (3.7 ± 1.5 s, P <0.01

and 4.2± 1.9, P <0.001), 50 (5.3 ± 1.5 s and 6.7 ± 2.0 s, P <0.001), 75 (6.7 ± 1.4 s and 8.7 ± 1.6 s, P <0.001), and 100 mg/kg (9.5 ± 2.3 s and 11.0 ± 1.5 s, P <0.001).

3.6. Activity of GBP and GPS on CCI induced static mechanical hyperalgesia

Unilateral sciatic nerve ligation elicited an elevated mechanical nociceptive paw withdrawal response duration (P< 0.001) from protocol day 7 till the end of experiment (on day 15). The hyperalgesia induced by gentle pin pressure on the operated hind paw was raised from the pre-ligation baseline of 0.5 s to 12.8 s on day 7 to 13.7 s on day 15th (Fig. 7). A significant main dose effect was afforded by GBP and GPS on the CCI-induced paw withdrawal threshold [time = (F (4, 175) = 427.0, P <0.0001), treatment = (F (6, 175) = 155.3, P <0.0001), interaction = (F (24, 175) = 21.1, P <0. 0001). Thus, GBP (100 mg/kg) reversed the CCI-induced augmented paw withdrawal duration (7.8 ± 1.8 s and 6.8 ± 1.7 s, P <0.001)1 and 3 h post-treatment on day 15 compared to CCI-vehicle treated animals 13.5 ± 1.5 s and 13.5 ± 2.3 s. GPS treatment also abolished CCI-evoked hyperalgesia to the pin prick stimuli at doses of 25 (11.2 ± 1.2 s, P <0.05), 50 (9.2 ± 2.6 s, 8.2 ± 2.3 s, P <0.001), 75 (6.5 ± 1.9 s, 5.2 ± 1.3 s, P <0.001) and 100 mg/kg (5.0 ± 1.8 s and 3.3 ± 1.2 s, P <0.001) on the 15th protocol day 1 and 3 h after treatment compared to CCI-vehicle control animals.

3.7. Activity of GBP and GPS on CCI-induced cold allodynia

There was a decelerated withdrawal reflex (P <0.001) to hind paw acetone application in the CCI animal group in comparison to sham operated controls. Accordingly, paw withdrawal duration in response to acetone was markedly increased from a pre-surgery baseline of 0.5 s (-5 day) to 12.2 ± 1.3 and 14.0 ± 1.3 on protocol days 7 and 15 respectively. This CCI induced increment in duration was subsequently diminished on protocol day 15 by GPS in a graded fashion by doses of 25, 50, 75 and 100 mg/kg to 12.0 ± 2.1 and 10.8 ± 2.0s (P <0.0001), 9.5 ± 1.4 and 8.7

± 1.5 s (P <0.0001), 7.0 ± 1.4 and 5.3 ± 1.2 s (P <0.0001), and 6.2 ± 1.3 and 4.2 ± 1.3 s (P <0.0001, P <0.0006) at 1 and 3 h post treatment. Likewise, a pronounced decrease in paw withdrawal duration (8.3 ± 1.2 s (P <0.0001) and 6.5 ± 2.7 s (P <0.0021) was also observed to GBP (100 mg/kg).

Both GBP and GPS had a significant main dose effect of CCI-induced cold allodynia [time = (F (4,175) = 480.6, P <0.0001), treatment = (F (6, 175) = 176.7, P <0.0001), interaction = (F (24, 175) = 20.83, P <0.0001)]. In the sham operated GPS/GBP treated animals there was also attenuation (P <0.0001) of cold allodynia when compared to the CCI controls (Fig. 8).

3.8. Activity of GBP and GPS on locomotor performance of rats

Ligation of the sciatic nerve of the left hind paw resulted in an overall decline in locomotor performance leading to a significant fall in the total number of lines crossed by the CCI-animals versus sham operated controls from days 7-15 (Fig. 9). Statistical analysis disclosed a significant main dose effect of GBP and GPS [time = (F (6,140) = 112.0, P <0.0001), treatment = (F (4, 140) = 15.1, P <0.0001), interaction = (F (24, 140) = 4.9, P <0.0001)]. *Post-hoc* analysis further revealed that the CCI-GPS animals experienced an increment in locomotor activity compared to the CCI-vehicle controls. The number of lines crossed by all the CCI animals decreased from a pre-surgery value of 67.6 ± 6.8 (-5 day) to 47.5 ± 15.9 (7 day) and 41.1 ± 19.4 (day 15, pretreatment). A marked increase in the number of lines crossed by the animals after treatment with GPS [(25 mg, 25.5 ± 2, 26.7 ± 3.3, P >0.05), (50 mg, 29.5 ± 4.5 and 29.5 ± 3.9, P <0.05), (75 mg, 40.3 ± 3.8 and 46.3 ± 2.6, P <0.001), (100 mg, 49.5 ± 5.5 and 52.8 ± 5.9, P <0.001)] was observed compared to the CCI-vehicle controls (20.7 ± 3.1 and 20.0 ± 3.7) at 1 and 3 h post treatment on day 15. GBP (100 mg, 34.7 ± 4.3 and 37.7 ± 2.9, P <0.001) treatment resulted in a decline in number of lines crossed compared to sham treated group (P <0.0001).

3.9. Activity of GBP and GPS on motor coordination

3.9.1. Rotarod

The effect of various doses of GPS (25-100 mg/kg) on motor coordination in the rotarod assay was performed. A significant main dose effect of GPS was observed [time = (F (6, 140) = 151.4, P <0.0001), treatment = (F (4, 140) = 455.3, P <0.0001), interaction = (F (24, 140) = 14.0, P <0.0001)]. Nerve ligation resulted in a deficit in motor coordination as evidenced by a drop in the dismount latency from 197.5 ± 15.1 s (day-5) to 156.5 ± 8.8 s (day 7) and 114.9 ± 6.0 s (day 15, pretreatment). Treatment of CCI rats with GPS at doses of 25, 50, and 75 mg/kg did not significantly alter the latency to dismount as 110.7 ± 6.2 s and 110.5 ± 4.4 s, 109.0 ± 7.01 s and 108.8 ± 2.48 s, 99.3 ± 7.25 and 110.7 ± 6.2 s compared to CCI-Veh 110.0 ± 4.7 and 107.7 ± 5.8 s on day 15 at 1 and 3 h post GPS administration. Although, GPS at 100 mg/kg dose revealed a fall in latency, 105.0 ± 7.4 s and 86.3 ± 4.6 s, more significant at h 3. On the contrary, GBP 100 mg/kg significantly reduced dismount latency to 85.8 ± 5.3 s and 80.0 ± 6.3 s compared to CCI-Veh treated animals, 110.0 ± 4.7 and 107.7 ± 5.8 s on day 15 at 1 (P <0.01) and 3 h (P <0.001) post treatment, respectively (Fig. 10).

4. Discussion

Neuropathic pain following nerve damage, whether from physical, chemical, metabolic, infection or other reasons, infrequently responds to conventional analgesics. The anti-epileptic agent GBP, however, has manifested consistent analgesic efficacy in animal models of neuropathic pain [9, 10] and also in patients with chronic pain of this type [39]. Several studies have shown that GBP is distinctively efficacious against allodynia and hyperalgesia [40]. It has also been demonstrated to be superior to morphine in relieving the static and dynamic components of allodynia [41] in addition to yielding a dose dependent elevation of withdrawal threshold to painful

stimuli in rodents [42]. Its clinical usefulness, however, is limited due to the occurrence of side effects, of which, dizziness, somnolence, ataxia and lethargy, are the most prominent dose limiting examples [41]. In consequence, there is a need for the development of drugs with an improved safety and efficacy profile for neuropathic pain. Nowadays, the concept of conjugating two or more drugs having different pharmacological activities, has received attention as a rationale to improve therapeutic index and minimize adverse effects [43]. In this context therefore, the antiallodynic and antihyperalgesic effects of GBP and its conjugation with the anti-inflammatory salicylaldehyde [29] (GPS), were investigated in a model of traumatic nerve injury (CCI) in male rats. A number of animal models have been reported to simulate human peripheral neuropathic conditions, a substantial proportion of which are based on procedures involving the sciatic nerve [43]. In the CCI technique, loose unilateral ligations of the sciatic nerve mimic many pathophysiological changes seen in patients with chronic neuropathic pain [21]. The model also possesses sensitivity to a number of systemically administered agents employed in the clinic for the symptomatic treatment of chronic neuropathic pain. Additionally, even topical application of GBP possesses effectiveness against allodynia and hyperalgesia in this model [38].

Our study demonstrated that intraperitoneal treatment with either GBP or GPS clearly diminished evoked nociceptive responses at the end of a 15-day protocol entailing the procedure. Systemic GBP is known to partially suppress mechanical allodynia and reverse thermal hyperalgesia, mechanical hyperalgesia and cold allodynia [44]. Efficacy of GBP against mechanical allodynia has been reported previously [41, 45] and in the current study, GBP presented distinct anti-allodynic potential following administration on day 15 of the CCI procedure and this concurs with previous reports. Analogously, GPS inhibited static allodynia in the rat model of CCI-induced neuropathic nociception. Hence, the paw withdrawal threshold increased in

a dose dependent manner with a mild rise at a dose of 25 mg/kg and a more pronounced effect at higher doses at the end of the protocol on day 15 of CCI procedure.

GBP has antihyperalgesic and antiallodynic properties and its mechanisms of action appears to be a complex synergy between augmented GABA synthesis, non-NMDA receptor antagonism and binding to the $\alpha_2\delta$-1 subunit of voltage dependent calcium channels [46]. Different sensory profiles might signify different classes of neurobiological mechanisms, and hence subgroups with dissimilar sensory profiles might react differently to treatment [17]. In our study, GPS produced an anti-allodynic effect which was evident from the extended nociceptive response latencies/thresholds after administration. Paw withdrawal latencies/thresholds of GPS treated animals were increased dose dependently and it is probable that the mechanisms involved were similar to those of GBP. It is well documented that thermal hyperalgesia as well as static allodynia are heralded by high threshold nociceptive afferents [47]. Though there are several underlying mechanistic possibilities [9], both systemic and intrathecal GBP have been reported to inhibit heat hyperalgesia induced by peripheral nerve injury [38].

The current study demonstrated that GBP reversed heat hyperalgesia and this decline potentially induced a rise in the paw withdrawal latency in heat hyperalgesia caused by CCI and this concurs with earlier studies [21, 38, 45, 48, 49]. Likewise, GPS produced a reversal of CCI induced thermal hyperalgesia and this was noticeably dose dependent. In a similar fashion, previous investigations indicated that GBP reduces mechanical hyperalgesia (pin prick response) [50, 51]. Here, we observed not only that GBP reduced the paw withdrawal duration in CCI rats but it did so dose dependently. Assessment of mechanical hyperalgesia after 1 and 3 h on day 15 of the CCI protocol divulged that GPS possesses prospective antihyperalgesic aptitude discernible from an enhanced withdrawal duration (s) compared to the ligated controls.

The CCI procedure in rats produces a characteristic hyperalgesic behavior in response to plantar application of acetone [52] and GBP has been reported to curtail cold hyperalgesia in CCI-operated rats [53-55]. We have also demonstrated that GBP treatment had an ameliorative effect on the enhanced sensitivity to the pin prick stimulus induced by the CCI procedure in the nerve ligated paw. In addition, GPS substantially reversed cold allodynia at higher doses because none of the animals reacted to acetone compared with CCI-drug naive animals. Consequently, it might be reasonably concluded that GPS virtually reduced cold allodynia modeled in the paradigm.

Chronic pain is often coupled with comorbidities such as depression and anxiety which may hinder daily activities imposing a major impact on quality of life [56, 57]. GBP has long been known to be effective in the treatment of anxiety like behavior [58] and it increases animal locomotor activity [38, 51] except at higher doses [1]. GBP 100 mg on its own not only negatively affected locomotor activity but it also further depressed the locomotor activity of the CCI group. In contrast, GPS had little effect on locomotor activity over the full dose range by itself, but did tend to reverse the locomotor depressant action of CCI.

On the accelerating rotarod, GBP *per se* has been shown to impair performance [1, 38, 59-62] and our findings are in full accord with these previous reports reflecting a motor deficit [22]. It was notable that GBP actually potentiated the degree of motor deficit caused by CCI and this may have conceivably been derived from a basic additive action. GPS on its own, by way of contrast, did not suppress motor coordination, even at higher doses, nor did it appear to modify the motor deficit induced by CCI [1, 22]. Our results also conformed to studies where GBP administration resulted in a trend towards impaired performance in CCI rats. However, GBP at lower doses did not decrease the latency to remain on a rotating drum [63]. On the other hand, GPS administration caused no deficits in motor function in CCI animals except for a milder effect at a dose of 100 mg at 3 h post administration. It may be plausible that an inherent anti-

inflammatory component of GPS facilitated mobility in CCI animals to a sufficient degree allowing the expression of improved open field locomotor activity and motor coordination on the rotarod.

There is evidence that the the efficacy of GBP declines with long-term use [64, 65] conceivably via down-regulation of the $\alpha_2\delta$-1 subunit of voltage-gated Ca^{2+} channels in the CNS [66]. In this context, we have observed the effect of GPS at 25 and 100 mg/kg after repeated daily administration over a 35-day protocol (See the supplementary data, S1). No decline in the efficacy was observed, hence implying that its activity does not decrease with extended use, indicating GPS as a prospective alternative to GBP, though further studies are warranted regarding this matter.

This investigation was conducted using males since a number of studies have recognized sex differences in response to pain and analgesics [67]. However, researchers are generally encouraged to consider gender in their study designs, collect data on both sexes (except when there is justification otherwise), analyze the data accordingly, and report the results in a complete and transparent way. Unless the effects of gender are studied, there will be inevitable gaps in the knowledge base, though in early non-clinical studies such as the present one, it is important that possible variability within findings is minimized [68, 69].

5. Conclusion

In conclusion, this is a preliminary study where the antiallodynic and antihyperalgesic properties of GPS have been assessed in relation to its possible side effects evaluated by the locomotor activity and rotarod paradigms. The outcome suggests that GPS not only possesses dose related efficacy against CCI induced allodynia and hyperalgesia, but it also has a lower propensity than GBP to initiate either motor discoordination or CNS depression. Correspondingly, the

potential utilization of GPS for alleviating neuropathic symptoms might be advocated, though further studies are merited.

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Author contributions

NA, FS and NI participated in research design. NA, MS, RU and NU conducted the experiments. NA and MS, SA and IU performed data analysis. NA, MUA, MK, and RDS wrote and contributed to the writing of manuscript.

Ethical Approval

All experiments were approved by the committee on animal research ethics, Department of Pharmacy, University of Peshawar. Endorsement for the study was granted under the registration # 10/ EC-15/Pharm.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary material related to this article can be found for this article in attachment as Appendix A.

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Fig 1

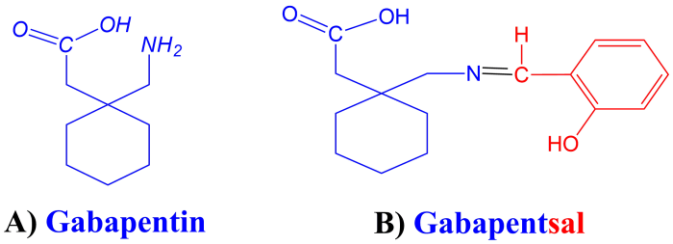

Fig 1: (A) Gabapentin**,** and **(B)** Gabapentsal

Fig 2

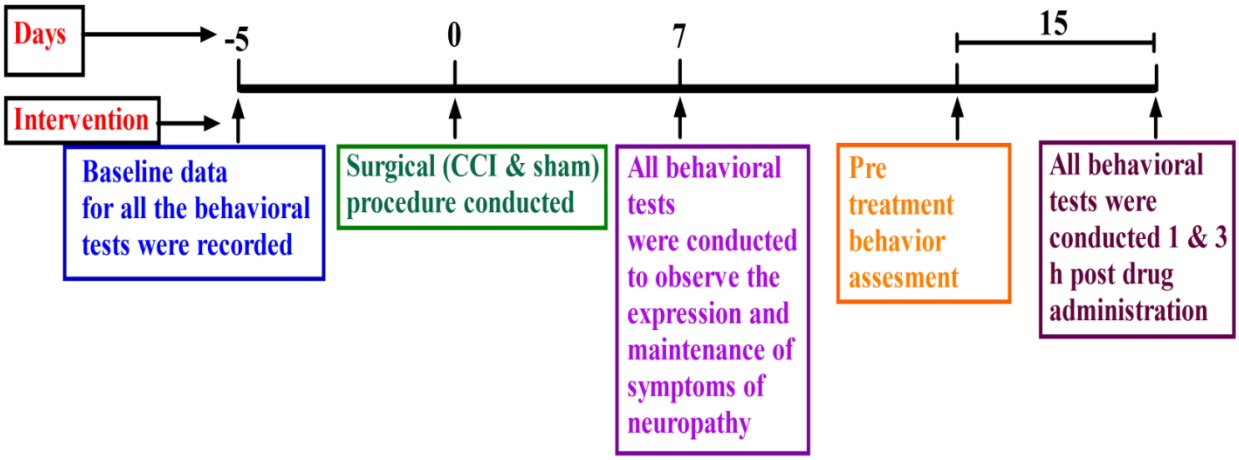

Figure 2: Experimental protocol

Fig 3

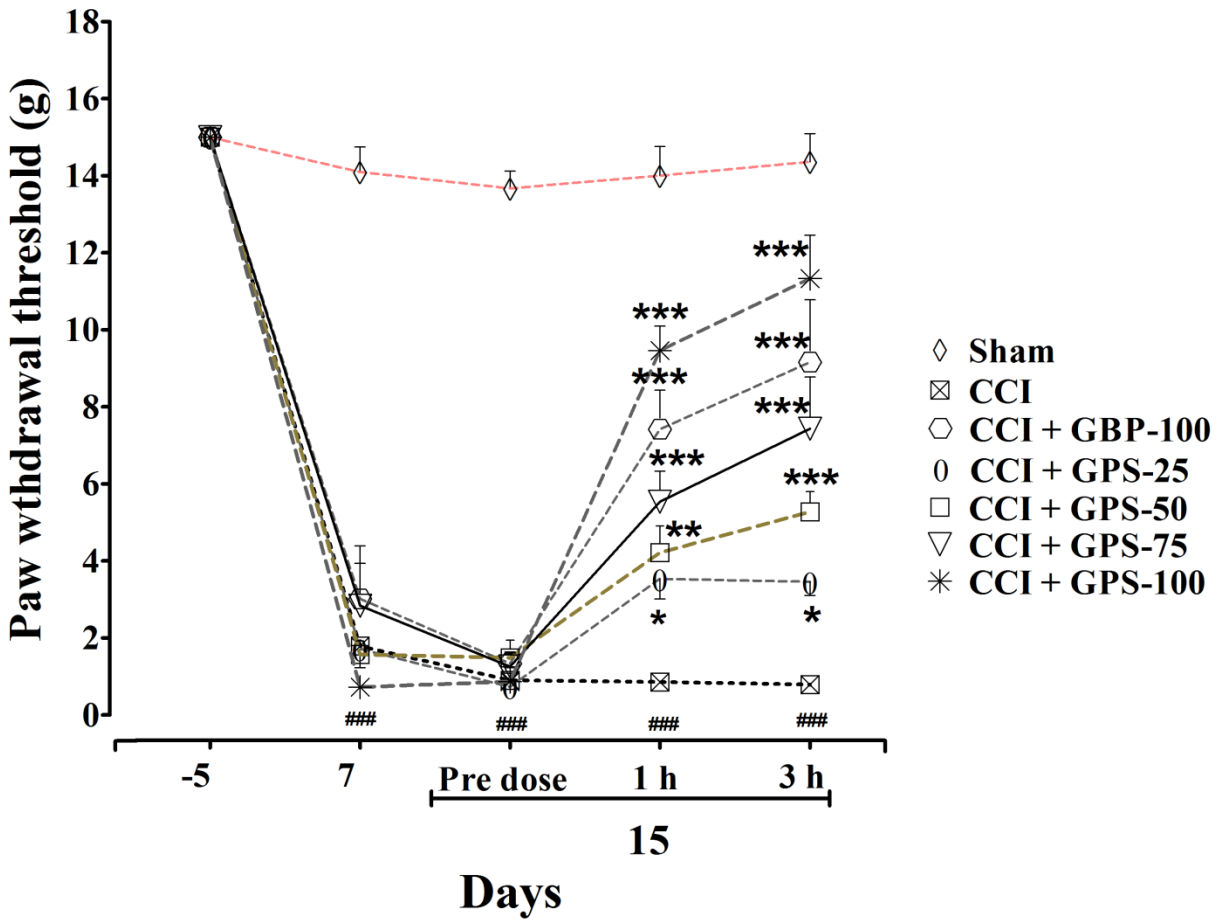

Figure 3: Effect of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on chronic constriction injury (CCI) induced static allodynia. Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated animals, *P <0.05, **P <0.01, ***P <0.001 compared to CCI-operated untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 4

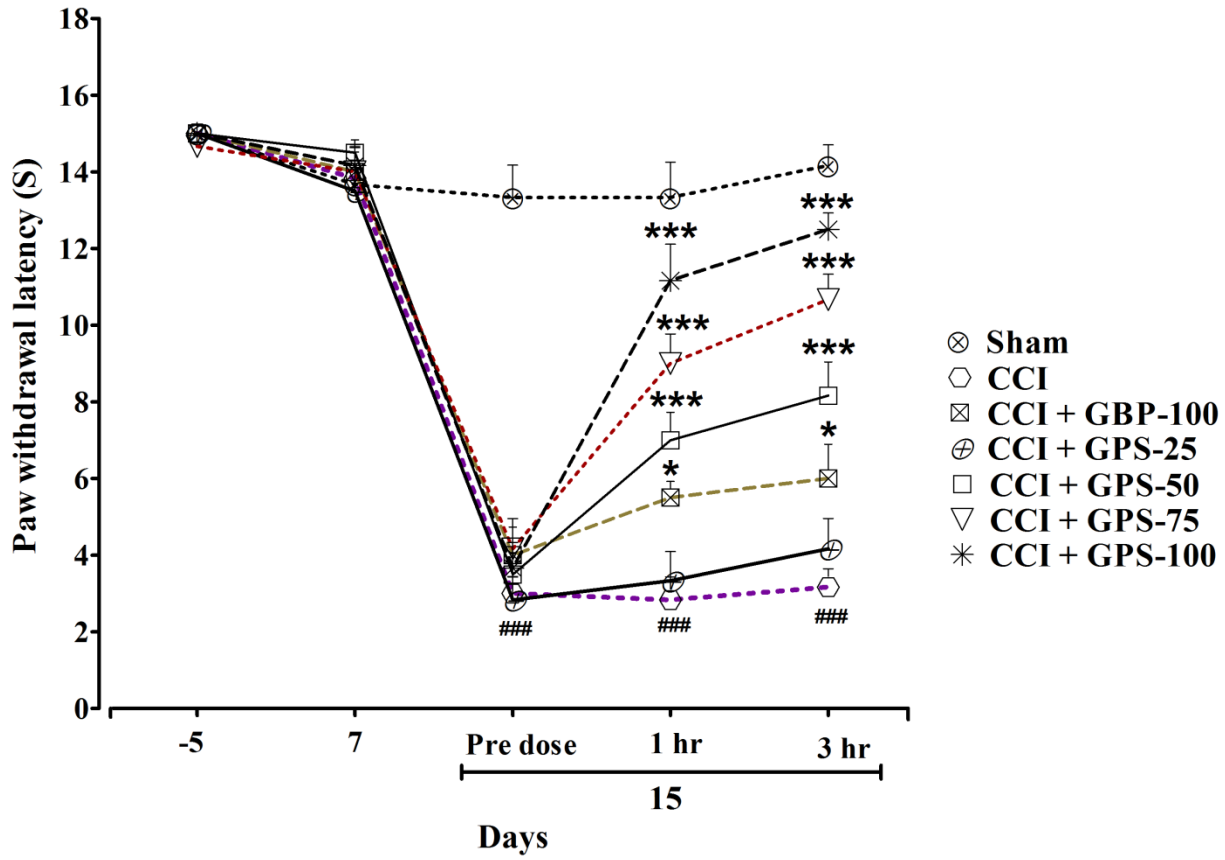

Figure 4: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50,75 and 100 mg/kg, i.p) on chronic constriction injury (CCI) induced dynamic allodynia. Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated animals, *P < 0.05, **P <0.01, ***P <0.001 compared to CCI-operated untreated controls, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 5

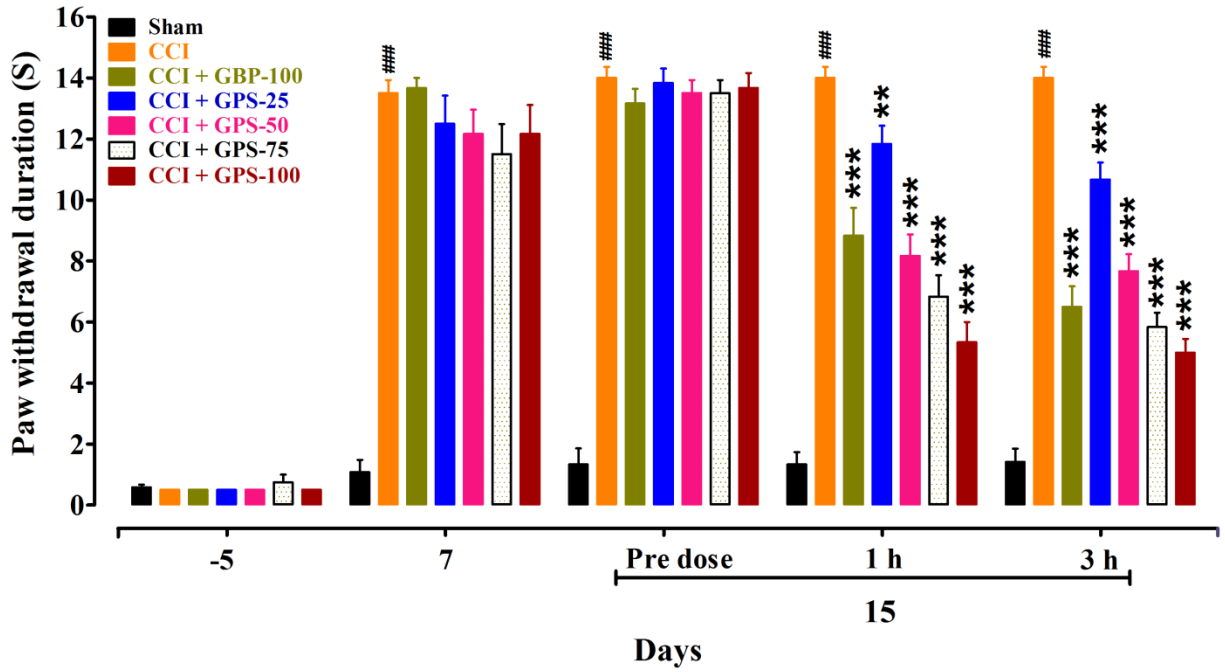

Figure 5: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on the chronic constriction injury (CCI) induced thermal hyperalgesia (paw withdrawal duration). Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated controls, $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001 compared to CCI-operated and untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 6

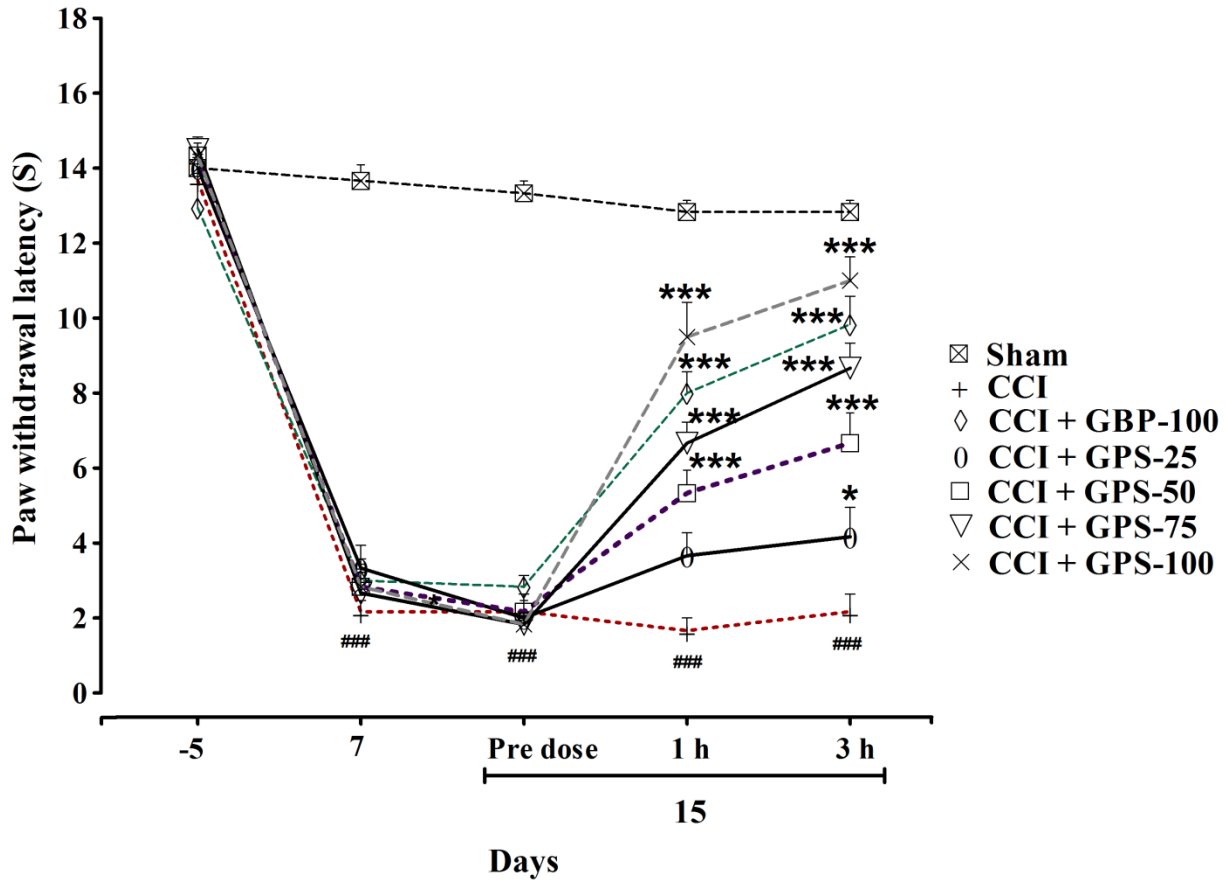

Figure 6: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on chronic constriction injury (CCI) induced heat hyperalgesia (paw withdrawal latency, PWL). Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated controls, $*P$ <0.05, $**P$ <0.01, $***P$ <0.001 compared to CCI-operated untreated controls, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 7

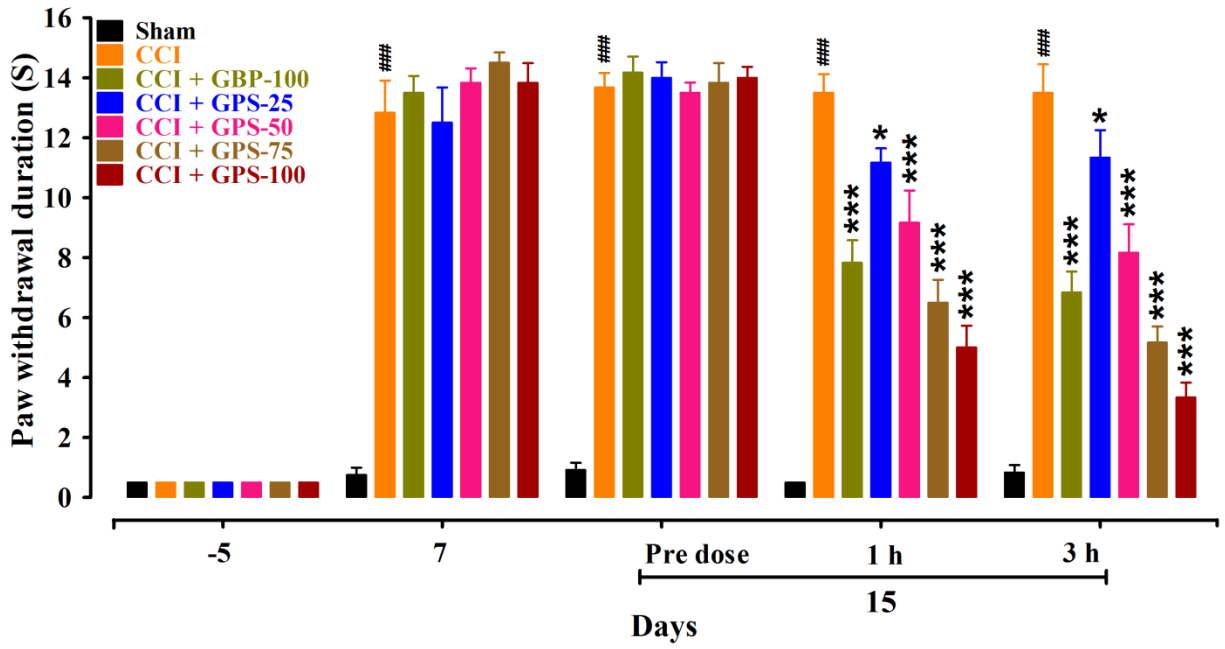

Figure 7: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on chronic constriction injury (CCI) induced static mechanical hyperalgesia [increased paw withdrawal duration (PWD). Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated animals, *P <0.05, **P <0.01, ***P <0.001 compared to CCI-operated untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 8

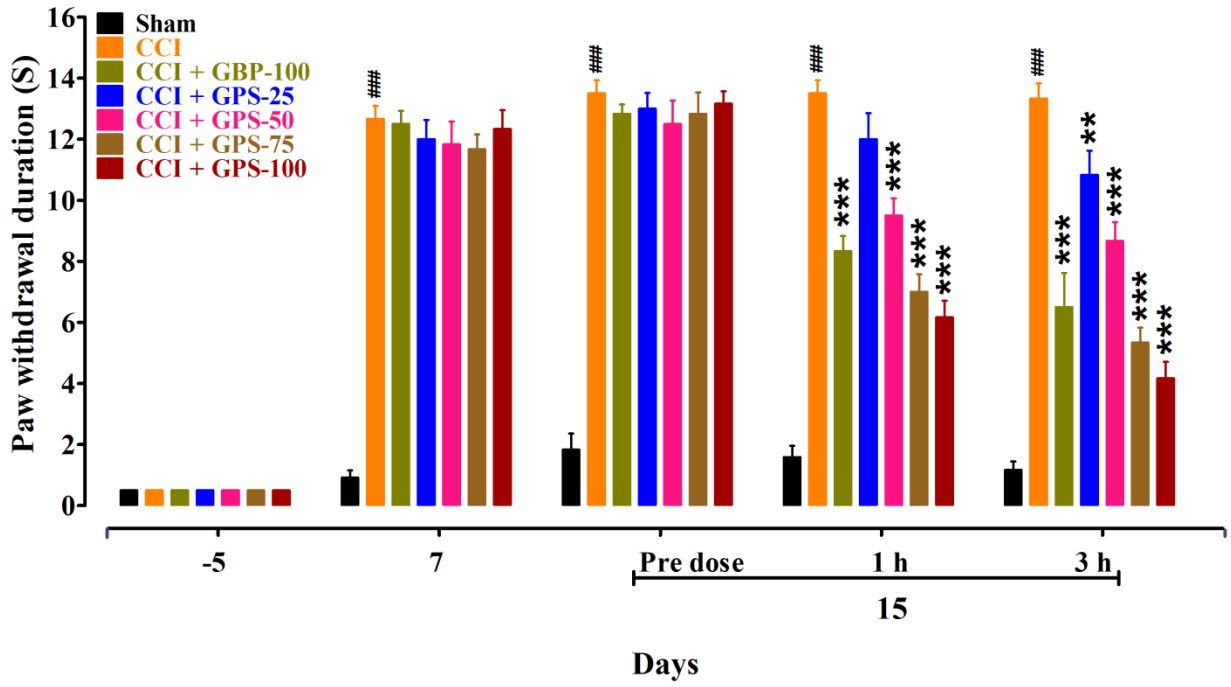

Figure 8: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on chronic constriction injury (CCI) induced cold allodynia [increased paw withdrawal duration (PWD) to a sprayed drop of acetone on the hindpaw]. Values are expressed as mean ± SD. $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated animals, *P <0.05, **P <0.01, ***P <0.001 compared to CCI-operated untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 9

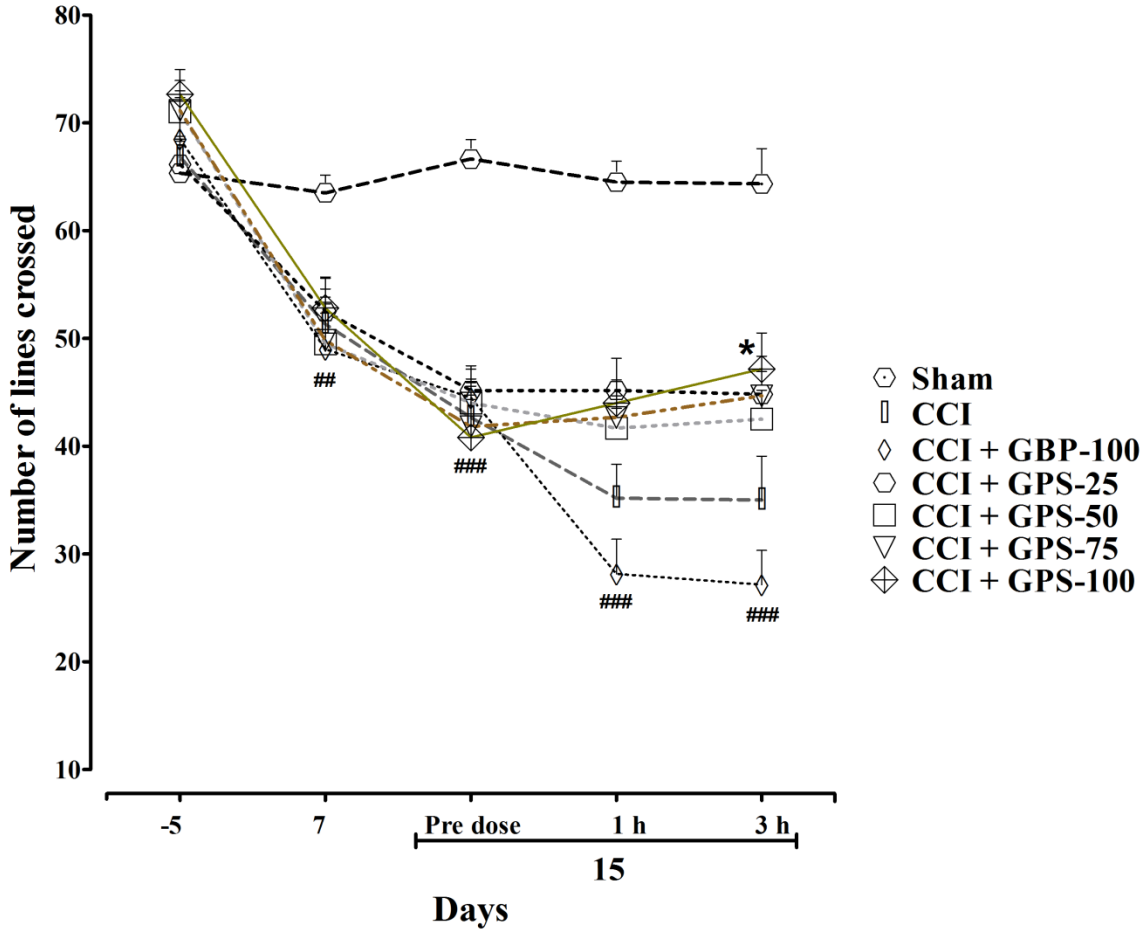

Figure 9: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on general locomotor activity in chronic constriction injury (CCI) animals expressed in a behavioral arena over a period of 20 min, on day 0, 7, 15 (pre drug treatment) and at 1 and 3 h post drug treatment on 15th day. Values are expressed as mean ± SD. $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated controls, $^{**}P$ <0.01, $^{***}P$ <0.001 compared to CCI-operated untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).

Fig 10

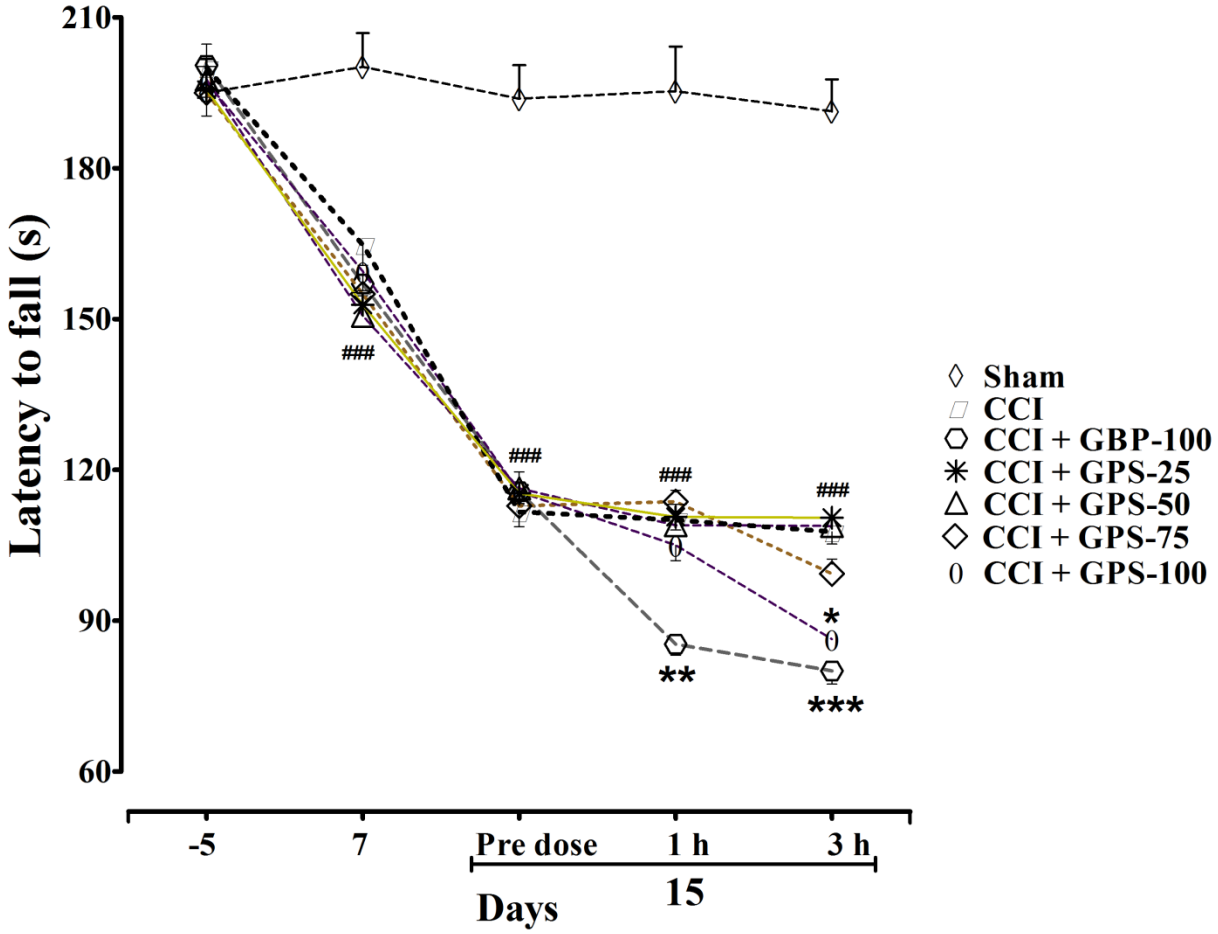

Figure 10: Activity of gabapentin [GBP] (100 mg/kg, i.p) and gabapentsal [GPS] (25, 50, 75 and 100 mg/kg, i.p) on rotarod performance in chronic constriction injury (CCI) animals expressed as the dismount latency on day 0, 7, 15 (pre-drug treatment) and at 1 and 3 h post drug treatment on 15th day. Values are expressed as mean ± SD. $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to sham-operated controls, **P <0.01, ***P <0.001 compared to CCI-operated untreated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni's analysis (n = 6).