Alcoholic extract of Banaba leaf prevents chronic constriction injury induced neuropathic pain in experimental animals

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ABSTRACT

Objective: Neuropathy always involves damage to the small nerve fibers. The present study was designed to investigate the effects of Banaba leaf extract on chronic constriction injury (CCI) induced neuropathic pain in experimental animals.

Methods: Wistar rats (200–250g) of either sex were used in the present study, they were divided into five groups - each containing six rats. Neuropathic pain was induced by chronic constriction injury (CCI) in rats. Pain was assessed by mechanical hyperalgesia, cold allodynia, and thermal hyperalgesia. Markers of oxidative stress, such as Lipid peroxidation and reduced glutathione were evaluated in sciatic nerve preparation. Banaba leaf extracts (50 and 100 mg/kg/p.o) were administered to the animals daily.

Results: Treatment with Banaba extract showed a significant and dose dependent increase in pain threshold in all the models. It also displayed significant reduction in Lipid peroxidation and an increase in reduced glutathione contents.

Conclusion: The present results showed the neuroprotective and antioxidant effects of Banaba leaf extract.

KEY WORDS: Neuropathic pain
Banaba leaf extract
Antioxidants

Introduction

Neuropathic pain is responsible for a significant amount of the morbidity associated with generalized and focal peripheral neuropathies [1]. Neuropathic pain commonly coexists with other types of pain such as low back pain associated with both radiculopathy and musculoskeletal abnormalities. The diagnosis of neuropathic pain is always challenging. Assessment of neuropathic pain should focus on identifying and treating the underlying disease processes and peripheral or central nervous system lesions, response to prior therapies and co morbid conditions that can be affected by therapy.

A number of agents belonging to different categories have been studied and used for the treatment of NP. The present allopathic treatment of NP is still difficult to look at new treatment and there is no single treatment that works for such conditions [2]. The clinical management of NP is often inadequate because of many reasons like inadequate diagnosis, inappropriate drug therapy, obsceneness in the pathophysiological pathways leading to NP. The existing strategies for treatment of NP can be broadly classified either as treatment based on pathogenetic concepts or based on the treatment [3]. Banaba plant, also called *Lagerstroemia speciosa* L., belong to the family "Lythraceae".

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Leaves of the plant contain different phytochemicals, which are reported to have a wide range of pharmacological activities such as antidiabetic, anti-oxidant, anti- inflammatory, anti-hypertensive, hepatoprotective, anti-obesity and analgesic. These evidences point that Banaba Leaf extract may have potential in neuropathic pain [4-7]. Till date, there is no evidence showing the use of Banaba Leaf extract in NP and hence the present study was designed for the preclinical evaluation of Banaba Leaf extract for analgesic activity and chronic constriction injury induced neuropathic pain.

Materials and methods

Drug and chemicals

Alcoholic extract of Banaba Leaf (AEBL) was procured from Kuber Impex Pvt. Ltd. Indore, with certificate of analysis. All others chemicals used in the study were of analytical grade and purchased from reputed supplier.

Experimental Animal

Wistar rats of either sex (200–250g) were used in the study. They were procured from Lachmi Biotech, Pvt. Ltd, Pune and were placed separately in polypropylene cages (approx. 4 per cage) with paddy husk as bedding. They were maintained under standard laboratory conditions (Temperature 23 ± 2^0 C, relative humidity 45 to 55 ± 10 %, 12:12hrs Light/dark cycles) throughout the experiments. The protocol was approved by Institutional Animal Ethic Committee (IAEC) of SSDJ College of pharmacy, Neminagar, Chandwad.

Study protocol

Animals were divided into following groups and each group consists of six animals. Group I: served as Sham operated (control) and received 1% carboxy methyl cellulose as a vehicle. Group II: served as disease control, neuropathic pain was developed by chronic constriction injury (CCI). Group III: CCI animals treated with AEBL (50 mg/kg, p.o) Group IV: CCI animals treated with AEBL (100 mg/kg, p.o), Group V**:** CCI animals treated with Pregabalin (10mg/kg, p.o). Neuropathic pain was developed after 9 days of surgery and the treatment was continued from 9th day to 30th day (21 days i.e three weeks).

Induction of neuropathic pain using CCI

Peripheral neuropathic pain was induced in rats by chronic constriction injury [8]. In brief, rats were deeply anesthetized with mixture of Ketamine and xylazine (8:2 in ml) 2ml/kg, i.p. The hair of the rat's lower back and thigh were removed using commercial surgical blade and the skin was sterilized with 0.5% chlorhexidine. The skin of the lateral surface of the right thigh was incised and cut was made directly through the biceps femoris muscle to expose the sciatic nerve and four ligatures were placed around the nerve proximal part of the trifurcation with an approximate distance of 1 mm between each ligature with non-absorbable sutures (catgut no 4-0). The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. After performing nerve ligation, muscular and skin layer were immediately sutured with silk thread. All surgical procedures were carried out under normal sterile conditions and were performed by the same investigator.

Assessment of neuropathic pain

Mechanical hyperalgesia

The mechanical hyperalgesia was assessed using pin prick test [9]. Rats were individually placed in a suspended acrylic chamber on a mesh floor. After the acclimatization period for 30 min, plastic filaments were applied perpendicularly to the planter surface of both hindpaw with sufficient force to bend the plastic filaments; paw withdrawal (lifting) latency was recorded in second. The reaction time was recorded in 0.5s units by a stopwatch. The cut off time of the paw withdrawal was 15s. A withdrawal time of more than 6s therefore is regarded as a positive response. The paw withdrawal time was measured weekly after confirmation of neuropathic pain.

Cold allodynia

The method was suggested by various authors. In that they dip the hind paw gently in ice cooled water and observed the paw withdrawal latency. In the present study we modified the method and used acetone to produce cold allodynia [10]. The cotton swab was deepened into fixed volume of acetone, it was then applied perpendicularly to the planter surface of both hind paws for measurement of the paw withdrawal (lifting) latency, The cut-off time was 15 sec.

Thermal hyperalgesia

The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded in 0.5 s units by a stopwatch. After each determination the tail was carefully dried. The cut off time of the immersion was 15 s. The withdrawal time of untreated animals was between 1 and 5.5 s. A withdrawal time of more than 6 s was regarded as a positive response [11].

Assessment of markers of oxidative stress

At the end of study the animals were sacrificed by euthanasia and the sciatic nerve was quickly isolated and transferred into cooled Tris hydrochloric buffered saline (pH 7.4). It was weighed on an Electronic Balance WENSAR, (Model - PGB200). The Sciatic nerve was cross-chopped with a surgical scalpel into fine slices. The tissues were then minced and homogenised in chilled Tris hydrochloride buffer (10mM, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10000 rpm at 0°C for 15 minutes using high speed cooling centrifuge (Remi C-24). The clear supernatant was used for the estimation of lipid peroxidation and reduced glutathione level [12,13].

Statistical Analysis

All the values are expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test as appropriate using computer based fitting program (GraphPad Prism version 5). Differences were considered to be statistically significant when $P<0.05$.

Results

Effects of AEBL on mechanical hyperalgesia, cold allodynia and Thermal hyperalgesia in CCL induced neuropathic pain.

The paw withdrawal latency in CCI rats after the induction of neuropathic pain was found to be significantly ($p<0.05$) decreased compared to sham operated rats. Significant ($P<0.05$) decreased in mean paw withdrawal latency was observed in the rats after one weeks of CCI as compare to control rats. Rats treated AELS 50 and 100mg/kg for 3 weeks significantly ($P<0.05$) and dose dependently increased the mean paw withdrawal latency when compared to CCI control rats. (Figure 1).

Figure 1. Effect of AEBL on mechanical hyperalgesia in CCI induced neuropathic pain. All values are presented as mean ± SEM, (n=6). $P<0.05^{\#}$ compared to control group. $P<0.05^{*}$ compared to CCI treated group.

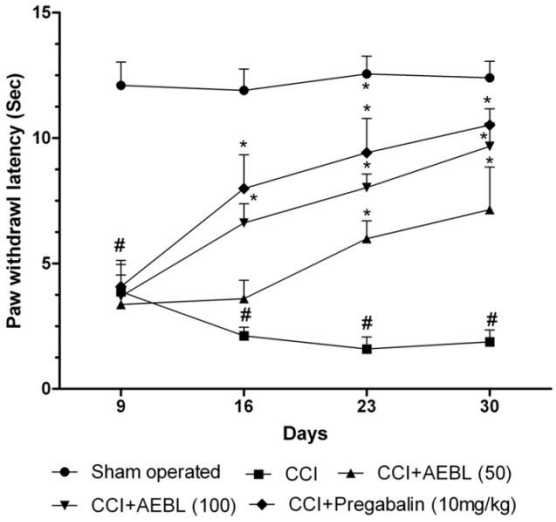
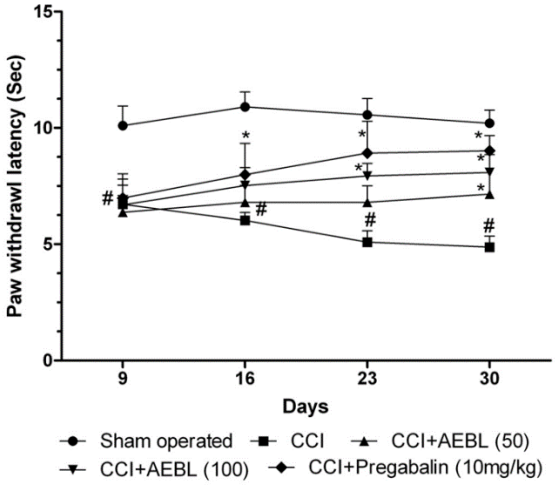


Figure 2. Effect of AEBL on cold allodynia in CCI induced neuropathic pain. All values are presented as mean ± SEM, (n=6). $P<0.05^{\#}$ compared to control group. $P<0.05^{*}$ compared to CCI treated group.



In cold allodynia, CCI induced neuropathic rats showed a significant ($P<0.05$) decreased in paw withdrawal latency up to the end of the study when compared to control rats. Oral treatment with AEBL (50 and 100mg/kg) and Pregabalin for 3 weeks showed significant ($P<0.05$) and a dose dependent increase in the changes in mean paw withdrawal latency when compared to CCI control rats. (Figure 2). In thermal hyperalgesia it was found that a significant ($P<0.05$) decrease in mean paw withdrawal latency was observed in rats with CCI compared to sham controlled rats. Rats treated with AEBL (50, 100mg/kg) and Pregabalin (10mg/kg) for 3 weeks significantly

(P<0.05) increased the changes in mean paw withdrawal latency compared to CCI control rats. (Figure 3).

Figure 3. Effect of AEBL on thermal hyperalgesia in CCI induced neuropathic pain. All values are presented as mean ± SEM, (n=6). P<0.05# compared to control group. P<0.05* compared to CCI treated group.

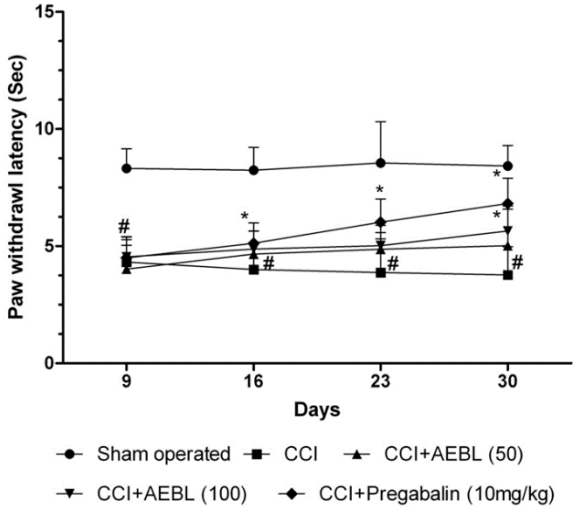
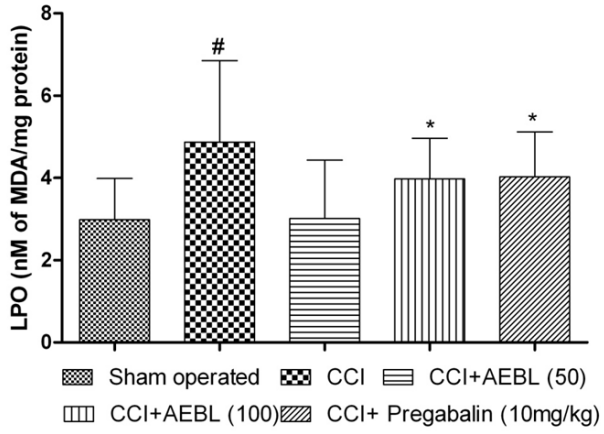


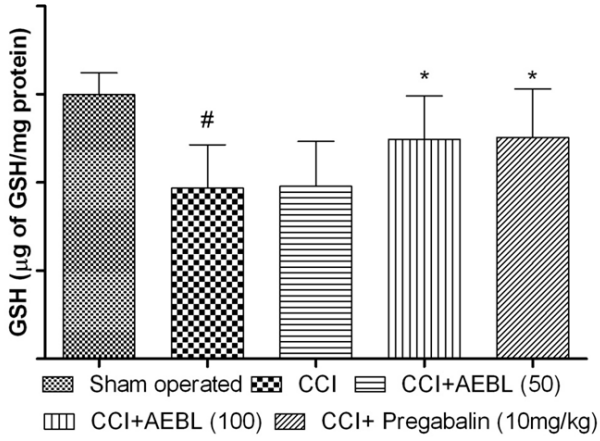
Figure 4. Effect of AEBL on Lipid peroxidation in CCI induced neuropathic pain. All values are presented as mean ± SEM, (n=6). P<0.05# compared to control group. P<0.05* compared to CCI treated group.



Effects of AEBL on markers of oxidative stress in CCL induced neuropathic pain.

Markers of oxidative stress such as Lipid peroxidation and reduced glutathione were evaluated in all the groups (Figure 4 & 5). CCI induced rats showed a significant (P<0.05) rise in lipid peroxidation whereas there was a significant (P<0.05) decrease in the level of reduced glutathione compared to sham operated animals. Treatment with AEBL for three weeks at 50 and 100mg/kg showed significant reduction in lipid peroxidation levels and increased in reduced glutathione level as compared to CCI rats.

Figure 5. Effect of AEBL on Reduced glutathione in CCI induced neuropathic pain. All values are presented as mean ± SEM, (n=6). P<0.05# compared to control group. P<0.05* compared to CCI treated group.



Discussion

Present study showed the beneficial effects of AEBL in CCI induced neuropathic pain in rats. AEBL was tested at two different doses i.e 50 and 100mg/kg/p.o for 21 days from the day on which pain developed (day 9). CCI is the most commonly used experimental model to induce neuropathic pain in rats as it mimics the various symptoms of chronic nerve compression in humans [14]. Several studies have reported the involvement of different mechanisms and pathways in the development of neuropathic pain. Free radical generation and oxidative stress has long been reported to be involved in the pathogenesis of neuropathic pain [10,15].

In the present study, neuropathic pain was evaluated using mechanical hyperalgesia, cold allodynia and thermal hyperalgesia. In all the three models there was a significant decreased in paw withdrawal threshold, indicated that CCI produced pain in the rats. These observations are in line with the previous study by Aswar *et al*, 2014 [16]. The altered levels of lipid peroxidation and reduced glutathione in CCI rats also indicated the involvement of oxidative stress in CCI induced neuropathic pain. Treatment with AEBL for 21 days shows significant alteration of paw withdrawal threshold in all the animals which indicates the strong analgesic activity of the drug. Furthermore, the treatment also showed a significant reduction in lipid peroxidation and reduced glutathione levels. Earlier studies reported the anti-diabetic and anti-oxidant activity of Banaba leaf extract [17-18]. It mainly contains

corosolic acid as an active phytoconstituent which is also reported to possess anti-diabetic, anti-oxidant, anti-inflammatory, anti-hypertension and analgesic activity [19].

In conclusion the present study showed the protective effect of AEBL in CCI induced neuropathic pain which needs to be evaluated further using different models of neuropathic pain.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgements

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