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Protective effect of co-administration of curcumin and sildenafil in alcohol induced neuropathy in rats

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ABSTRACT

Neuropathic pain associated with chronic alcohol consumption is a medico-socioeconomical problem that affects both central and peripheral nervous system and has no satisfactory treatment till date. The present study was designed to investigate the protective effect of co-administration of curcumin and sildenafil on alcohol induced neuropathic pain in rats. In order to carry out this, ethanol (35% v/v, 10 g/kg, p.o.) was administered for 10 weeks to induce neuropathic pain. Curcumin (30 and 60 mg/kg, i.p.) and sildenafil (5 and 10 mg/kg, i.p.) were given alone and in combination at their lower doses (30 mg/kg curcumin and 5 mg/kg, sildenafil, i.p.) to investigate the changes in thermal and mechanical hyperalgesia, allodynia and histopathological parameters. Biochemical estimations of thiobarbituric acid reactive species, glutathione and protein was also carried out to evaluate oxidative stress. The results revealed that chronic alcohol consumption for 10 weeks caused significant thermal and mechanical hyperalgesia, allodynia and increased oxidative stress. Individual administration of both the drugs at their low as well as high doses were able to improve the symptoms of alcohol induced neuropathic pain. Whereas co-administration of curcumin and sildenafil at their lower doses itself were found to significantly improve nerve functions, biochemical and histopathological parameters as compared to their individual administration. It is therefore proposed that co-administration of curcumin and sildenafil may bring new dimension towards attenuation of alcohol induced neuropathic pain affecting central as well as peripheral nervous system.

1. Introduction

Neuropathic pain is caused due to damage of neurons, direct damage to the nervous system or disease of somatosensory nervous system (Pasero, 2004; Costigan et al., 2009; Dworkin et al., 2010; Xu et al., 2012). It is characterized by allodynia, hyperalgesia, and paraesthesia (Shields et al., 2003; Campbell and Meyer, 2006; Grovle et al., 2013). Peripheral neuropathic pain is most commonly reported aberration in patients with chronic alcoholism (Dina et al., 2007; Misra and Kalita, 2009; Ferrari and Levine, 2010). Painful peripheral polyneuropathy, Wernicke encephalopathy, cortical and motility dysfunction, psychosis and delirium tremens are some of the neurological aberrations reported in chronic alcoholic patients (Yerdelen et al., 2008; Kandhare et al., 2012). In addition, chronic alcohol consumption produces a sustained increase in stress hormones, epinephrine and glucocorticoids, that are exacerbated through withdrawal of alcohol (Dina et al., 2008; Fu et al., 2015). Alcohol is a well-known promoter of oxidative stress by decreasing endogenous antioxidants like α-tocopherol, ascorbate and vitamin E concentration (McDonough, 2003; Kandhare et al., 2012). Moreover, it is reported that neuropathic effect of alcohol is exerted either *via* direct damage to DNA, cellular protein and lipid or, indirect damage to signaling pathway that regulates oxidative stress and exerts vicious effects (Cheesman, 1993; Kandhare et al., 2012). This causes significant decrease in velocity of nerve conduction, impairment in neural function, changes in vascular permeability and endoneural hypoxia (McDonough, 2003; Kandhare et al., 2012). Combination of gabapentin and extended release morphine, gabapentin and extended release oxycodone, pregabalin and oxycodone, pregabalin and lidocaine (5% w/w, topical) as well as sodium valproate and nitroglycerin (spray) (Xu et al., 2012) are reported to provide satisfactory pain relief with reduced side effects. Despite several efforts made in past to investigate the effect of synthetic drugs in peripheral neuropathy, significant clinical success has been limited and successful therapy is still a farfetched dream (Gloria et al.,

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1997; Peters et al., 2006).

Curcumin, an alkaloid from plant *Curcuma longa*, is widely used as an additive in Indian food. Its antioxidant, antiarthritic, antirheumatic, analgesic and gastro-protective effects are well documented (Ahmad, 2013; Díaz-triste et al., 2014; Zanjani et al., 2014). It is reported to have inhibitory effect over mitogen protein kinase (Jeon et al., 2013), cyclooxygenase 2 (COX-2) pathway and neuropathic pain induced by diabetes (Li et al., 2013). The antidepressant effect of curcumin is also reported by increasing serotonin, norepinephrine and dopamine levels in the brain that inhibit monoamino oxidase (Sanmukhani et al., 2013). Sildenafil is (mainly) known mainly for the treatment of erectile dysfunction (Boolell et al., 1996). It is also reported to have anti-depressant and gastro-protective effects (Díaz-triste et al., 2014). It has been found to alleviate insulin sensitivity *via* attenuating oxidative stress (Aboryag et al., 2013) and inhibit selective phosphodiesterase-5 (Patil et al., 2004). The use of sildenafil in neuropathic pain is well reported (Huang et al., 2010; Wang et al., 2015).

In the previous study, curcumin has been reported to attenuate alcohol induced neuropathic pain in rat model (Kandhare et al., 2012). As mentioned above, sildenafil has been reported in reduction of neuropathic pain in rat model (Huang et al., 2010). In the present study, an effort has, therefore, been made to investigate the advantages of co-administration of curcumin and sildenafil at their sub-effective doses over their individual treatments on ethanol induced neuropathic pain. It is important to note that the protective response of co-administration of curcumin and sildenafil for attenuation of alcohol induced neuropathy in rats has been reported for the first time.

2. Material and methods

2.1. Drugs and chemicals

Curcumin was procured from Central Drug House (CDH) Pvt. Ltd., India. Sildenafil was gifted by Mankind Pharmaceuticals Pvt. Ltd., Baddi, India. Protein kit was procured from Erba Mannheim, Trichloroacetic acid, and EDTA from Loba Chemie and Thiobarbituric acid from Himedia Labs.

2.2. Animals

Wistar albino rats of either sex, weighing between 180–270 g, were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Animals were housed in central animal house facility, Lovely Institute of Pharmacy, Lovely Professional University, Punjab, India, under standard laboratory conditions, at 25 ± 2 °C and 12 h light and dark cycle. They were given free access to rat chow diet and water *ad libitum*. Before conducting experiment, animals were acclimatized to laboratory conditions for seven days.

The protocol (LPU/LSPS/IAEC/CPCSEA/MEETING NO.1/ SEPTEMBER 2015/16PROTOCOL NO.5) was approved by Institutional Animal Ethics Committee, Lovely Institute of Pharmacy, Lovely Professional University, Punjab, India.

2.3. Induction of neuropathy using alcohol and treatment schedule

In order to carry out this study, total 60 animals were taken and randomly divided into 10 groups, each group containing six animals. The study design is shown in Table 1. Alcoholic neuropathy was induced by administration of 35% (v/v) ethanol (10 g/kg, twice daily through oral gavage) in double distilled water for 10 weeks (Kandhare et al., 2012; Sarifakioglu et al., 2004; Zhu et al., 2014). All the animals of group V to X were administered with the given dose as per study design followed by oral administration of 35% (v/v) ethanol after 1 h. Dosing was carried out up to 10 weeks and all the behavioral assays were performed by a blind observer on 1st, 2nd, 4th, 6th, 8th, and 10th week.

Table 1
Induction of neuropathy and treatment schedule.

Groups	Treatment	Doses
Animals without ethanol administration		
I	Normal Control (N)	–
II	Vehicle (V)	20% DMSO, i.p.
III	Curcumin *per se* (CRM)	60 mg/kg, i.p.
IV	Sildenafil *per se* (SD)	10 mg/kg, i.p.
Animals with ethanol Administration		
V	Experimental control/ ethanolic control (EC)	10 g/kg, 35% v/v, bis in die, p.o
VI	Curcumin low dose+ethanol (CRML+E)	30 mg/kg, 35% v/v bis in die, p.o
VII	Curcumin high dose+ethanol (CRMH+E)	60 mg/kg, 35% v/v bis in die, p.o
VIII	Sildenafil low dose+ethanol (SDL+E)	5 mg/kg, 35% v/v bis in die, p.o
IX	Sildenafil high dose+ethanol (SDH+E)	10 mg/kg, 35% v/v bis in die, p.o
X	Curcumin low dose+sildenafil low dose+ethanol (CRML+SDL +E)	30 mg/kg (curcumin)+5 mg/kg (sildenafil), 35% v/v bis in die, p.o

2.4. Behavioral examinations

2.4.1. Motor coordination test (Rota rod test)

Motor coordination test (grip muscle strength) was evaluated using Rota-rod apparatus as described by Muthuraman et al., (2008, 2011). Rats were placed on the rotating rod and evaluated for the falling time from the roller with a cut off period of 1 min.

2.4.2. Paw heat hyperalgesia (Hot plate test)

Thermal hyperalgesia was assessed in terms of thermal nociceptive threshold using Eddy's hot plate as described in previous reports (Eddy et al., 1950; Kaur et al., 2010; Muthuraman et al., 2011). The plate was preheated and maintained at a temperature of 52.5 ± 2.0 °C. Rats were placed on the hot plate and nociceptive threshold was recorded in seconds by observing the licking of hind paw. The cut-off time of 20 s was maintained.

2.4.3. Paw heat allodynia test

Heat allodynia of the hind paw was assessed using Eddy's hot plate by recording the reactivity to non-noxious thermal stimuli (Eddy et al., 1950; Muthuraman and Singh, 2011). For testing allodynia, rats were placed on the top of a controlled preheated plate (maintained at 45 ± 0.5 °C). The degree of the nociceptive threshold was measured by the left hind paw withdrawal response of rats. The cut-off time of 30 s was maintained.

2.4.4. Paw cold allodynia test (Acetone drop test)

The reactivity of hind paw to non-noxious cold chemical stimuli was evaluated using acetone drop test as described by Muthuraman et al. (2011). In brief, 100 µl of acetone was sprayed on the plantar surface of hind paw of rat. Duration of withdrawal reaction was observed in the form of licking, shaking or rubbing of hind paw for a maximum of 20 s.

2.4.5. Mechanical hyperalgesia (Pin prick test)

Mechanical hyperalgesia was evaluated using the method as described by Kaur et al. (2010) with certain modifications. The surface of the injured hind paw was touched with the point of the bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response in normal non-operated animals, but the intensity was controlled in such a way that it shouldn't penetrate the skin. Withdrawal of hind paw was recorded as indicative of nociceptive threshold with a maximum cut-off time of 20 s.