Anti-Nociceptive Activity of Aqueous Licorice Root Extract on Neuropathic Pain and its Effect on some Selected Biochemical Parameters in Male Wistar Rats

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ABSTRACT

Licorice (Glycyrrhiza glabra) is a traditional medicinal, sweet and soothing herb known for its anti-inflammatory effect in painful conditions. Neuropathic pain is associated with certain set of symptoms, increased drug prescriptions and regular visits to health care providers. In the present study, we examined the effect of licorice on chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain. Neuropathic pain was induced by CCI of sciatic nerve in 6-week-old male albino rats for 3weeks. Paw withdrawal thresholds were assessed on day 3, 7, 14 and 21 using von Frey test after which serum levels of cortisol, brain lactate dehydrogenase (LDH) and brain nitric oxide (BNO) were evaluated using ELISA kit.Groups treated with low dose licorice post-surgery treated (LDL), high dose licorice post-surgery treated (HDL), low dose licorice pre-surgery treated (LDLp), high dose licorice pre-surgery treated (HDLp) and imipramine demonstrated significant increase in change of paw withdrawal threshold compared with ligated control. Serum levels of Cortisol, brain LDH and BNO were significantly reduced in HDL, LDLp and HDLp treated groups when compared with ligated control. Our result shows that licorice extract demonstrates anti-nociceptive activity by reducing the serum level of cortisol, brain LDH and BNO and the effect is dose and duration dependent.

Keywords: Brain, Lactate, Dehydrogenase, Nitric, Oxide, Cortisol

INTRODUCTION

Neuropathic pain is the most common cause of chronic pain worldwide,[1] causing a lot of psychological discomfort, "physiologic stress" and physical disability challenges, which translates to high medical burden on the global economy.[2] Neuropathic pain occurs as a result of trauma to the nerve or diseased nerve conditions.[3] It is commonly present in patients with cancer, multiple sclerosis, long-standing diabetes mellitus, phantom limb pain, post-mastectomy, HIV/AIDS, spinal stenosis, stroke and old age.[4] Its classical presenting symptoms are hyperalgesia, allodynia, paresthesia and spontaneous pain.[5]

Glycyrrhiza *glabra* is native to Southern Europe and certain parts of Asia such as China and India. The root extract of Glycyrrhiza *glabra* has been used in many food products, soft drinks and snacks as a sweetener.[6] Several studies have demonstrated the pharmacological importance of Glycyrrhiza *glabra* such as anti-inflammatory,[7] anti-tumour,[8] anti-viral,[9] anti-nociceptive activities on acute pain[10] and anti-microbial[11] activities among others.

Cortisol is known to play a major role in stress response to pain perception.[12] Stress has been proved to play a pivotal role in the transition of acute to chronic pain via the dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis.[13] The essence of this rise in serum cortisol level in the acute phase is to facilitate the release of energy and substrate necessary to cope with stress physiologically.[14] However, prolonged surge in serum cortisol results in conversion of its anti-inflammatory property to pro-inflammatory effect due to HPA axis dysfunction,[15]thereby exaggerating pain severity.

Likewise, lactate dehydrogenase (LDH) is a cytoplasmic cellular enzyme present in all organs of the body where it reversibly converts lactate into pyruvate, thus linking the glycolytic and oxidative metabolism energy generating pathways.[16] However, lactate is being considered as a metabolic fuel in the brain. Under normal physiologic condition, L- lactate supplies up to 10% of the brain metabolic need, which can increase up to 60% in high metabolic conditions like severe hypoglycemia, hypoxia

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and intense physical exercise.[17] It serves as a pointer of cellular integrity imbalance[18] and has been implicated in panic disorder psychiatry illness.[19] Further, nitric oxide (NO) and its associated enzymes are also involved in many physiological[20] and pathophysiological processes like pain modulation.[21]

It is apparent there are different treatments for neuropathic pain, and they have their limitations.[22] Natural substances are becoming more common in replacing established medications for managing neuropathic pain. In furtherance of our interest in neuropathic pain, we investigated the effect of licorice root extract on chronic constriction injury of the sciatic nerve to induce neuropathic pain.

MATERIALS AND METHODS

Animals

Thirty-five 6-week-old male Wistar rats weighing between 200-250g were used for the study. The rats were housed and maintained to acclimatize for 1week in standard conditions andwere given free access to rat pelleted diet and water in the Animal House, College of Medicine, Ekiti State University. The study was carried out in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals.[23]

Animal groupings

The animals were randomly assigned to one of the following experimental groups (n = 5 per group), ligated and were treated accordingly.

Group I: received distilled water (10ml/kg, orally) daily; designated as non-ligated control.

Group II: received distilled water (10ml/kg, orally) daily; designated as ligated control.

Group III: received reference drug (40mg/kg, orally); designated as imipramine treated.

Group IV: post-surgery treated with low dose licorice extract (75mg/kg, orally); designated as low dose treated (LDL).

Group V: post-surgery treated with high dose licorice extract (150mg/kg, orally); designated as high dose treated (HDL).

Group VI: pre surgery treated with low dose licorice extract (75mg/kg, orally); designated low dose pre-treated (LDLp).

Group VII: post-surgery treated with high dose licorice extract (150mg/kg, orally); pre surgery; designated high dose pre-treated (HDLp).

Neuropathic pain was induced in groups II to VII. Groups I and II received no interventions. Administrations of treatment (extract and reference drug) began in groups III, IV, and V three days after surgery and continued for 18 days. Group III received 10mg/kg of Imipramine; IV and V received 75mg/kg and 150mg/kg of licorice extract respectively. Groups VI and VII received 75mg/kg and 150mg/kg respectively, for 10 days before surgery and treatment continued three days after surgery for another 18 days.

Extract preparation

Licorice root powder was purchased from Amazon and was sold by Herbs and Crops Overseas, India with batch no: LRP-2017/02. A portion of the powder (50 g) was mixed with 100ml of sterile distilled water in a flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filter paper and kept in an airtight amber colored container.[24]

Induction of neuropathic pain

Chronic constriction injury (CCI) of the sciatic nerve was used to assess neuropathic pain.[25] Rats were anesthetized using sodium pentobarbital (40mg/kg) via intraperitoneal (i.p.) administration. Neuropathic pain was thereafter induced by chronic constriction (CCI) of the sciatic nerve using a suture. An incision about 3cm long was made into the skin that overlies the area between the gluteus and biceps femoris muscles, and the common sciatic nerve of the right hind paw was exposed at the mid-thigh level. The suture was tightly tied around the sciatic nerve making a diameter of approximately $^{1}/_{3}$ - ½ mm. After the surgery, the animals were allowed to recover under antibiotic cover.

Von Frey Filament Test

Von Frey Filament test was used to assess static allodynia.[26] Briefly, rats were placed in a suspended chamber that has wired mesh floor. They were allowed to acclimatize

for 20 minutes. Planter surface of left hind paws were tested using von Frey Filament Test; hair and paw withdrawal thresholds were recorded. This test was done before surgery on the rats and on days 3, 7, 14, and 21 post-surgery.

Determination of biochemical parameters

At the end of the treatment period, the rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; i.p., BDH chemicals Ltd., Poole, England). Blood samples were obtained from cannulated carotid artery into heparinized centrifuge tubes. Plasma was extracted by centrifugation at 3000 rpm for 15min. Brains were quickly removed, washed in cooled 0.15M NaCl and were then homogenized in 2ml of ice-cold potassium phosphate buffer (0.1M, pH:7.4) using an improvised homogenizer. Samples were centrifuged at 5000 rpm for 15 min to obtain the supernatant. The homogenate obtained was stored at -20 degree Celsius until the time of biochemical analysis. Serum level of cortisol, brain NO and brain LDH were determined using an Enzyme Immunoassay (EIA) kit from Randox laboratory Ltd. Co (Antrim, UK).

Statistical Analysis

All data are expressed as means ± standard error of the mean (SEM) for 5 rats per group. Statistical group analysis was performed with graph pad statistical software (Graph Pad Inc., San Diego, CA, USA). Test of variance was done using ANOVA, followed by Tukey's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$.

RESULTS

Von Frey Filament Latency Test

Pain threshold of ipsilateral hind paw of animals using von Frey filaments across the groups is shown in table 1. At baseline there was no significant change in paw withdrawal threshold. On day 3, there was significant increase in paw withdrawal threshold in HDLp treated group when compared only to ligated control. By day 7, HDL, LDLp and HDLp treated groups demonstrated significant increase in threshold compared to ligated control, LDLp and HDLp treated groups also demonstrated significant increase in threshold compared with imipramine treated group. Only HDLp treated group demonstrated significant increase in threshold compared with LDL treated group. Furthermore, on day 14 and 21 HDL, LDLp and HDLp treated groups all demonstrated significant increase in threshold compared with the two control groups, imipramine and LDL treated groups.

Serum cortisol concentration

Figure 1 showed serum cortisol concentration in all the groups. There was significant increase in serum cortisol level of ligated control, imipramine treated, LDL treated, HDL treated and LDLp treated groups when compared with non-ligated control group. Moreover, LDLp and HDLp treated groups showed significant decrease in serum cortisol concentration compared with ligated control, imipramine and LDL treated groups.

Brain Lactate dehydrogenase (LDH) concentration

Figure 2 shows the effect of licorice on brain LDH concentration in all the groups. There was significant increase in brain LDH concentration in ligated control, imipramine, LDL, HDL and LDLp treated groups when compared with non-ligated control group. Moreover, HDL, LDLp and HDLp treated groups had significant reduced LDH concentration when compared with ligated control group. HDL and HDLp treated groups also demonstrated significant reduced brain LDH concentration when compared with LDL treated group. HDLp also showed a significant decrease in LDH concentration when compared to imipramine treated group.

Brain nitric oxide (BNO) concentration

Changes in BNO concentration level among the groups is shown in figure 3. Imipramine, HDL, LDLp and HDLp treated groups all demonstrated a significant decrease in BNO concentration when compared with ligated control. Moreover, BNO concentration was significantly reduced in HDLp group when compared with normal control and LDLp groups. Only ligated control group showed significant increase in concentration when compared with non-ligated control group.

Table 1: Effect of the aqueous licorice root extract administration on pain threshold in animals using von Frey test.

	(Von Frey) Force (g)				
Rat groups	**Base line**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Control	28.1±1.1	27.7± 0.4	26.7 ± 0.4	27.3 ± 0.4	25.9 ± 0.2
Control ligated	28.9 ± 0.7	12.7± 1.7 [a]	13.3 ± 1.1 [a]	14.1 ± 1.1	14.5 ± 1.0
Imip treated	27.3± 0.4	19.7 ±2.5	15.1± 0.2	27.9 ± 1.2	35.9 ± 4.4 [b]
LDL treated	27.3 ± 0.4	19.7± 2.7	24.1 ± 2.1	27.7 ± 1.3	39.7 ± 5.7 b
HDL treated	27.7± 0.4	26.9± 0.4 [b]	26.3 ± 0.2 [b]	45.3± 6.6 [a b c d]	56.1 ± 1.6 [a b c]
LDLp treated	27.7 ± 0.4	26.5 ± 0.6 [b]	35.5 ± 4.6 [b c]	51.9 ± 5.0 [a b c d]	59.9 ± 0.2 [a b c d]
HDLp treated	28.1± 0.4	32.1 ± 2.1 [b c]	40.9 ± 5.1 [a b c d e]	55.7 ± 1.3 [a b c d]	68.1 ± 7.8 [a b c d]

Data expressed are means ± SEM, n = 5. ([a]p?0.05 vs control, [b]p<0.05 vs Ligated control, [c]p<0.05 vs imipramine treated, [d]p<0.05 vs LDL treated, [e]p?0.05 vs HDL treated).

Key: Imipramine (Imip); Low dose licorice (LDL); High dose licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

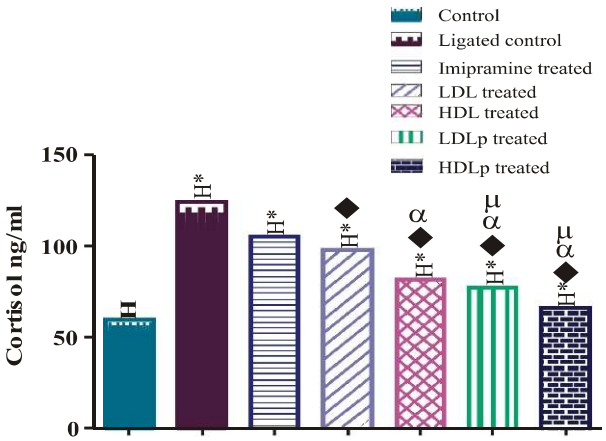

Figure 1: Effect of Licorice on serum cortisol concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, [?]p<0.05 vs ligated control, [α]p<0.05 vs imipramine, [μ]p<0.05 vs Low dose licorice (LDL); High dose Licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

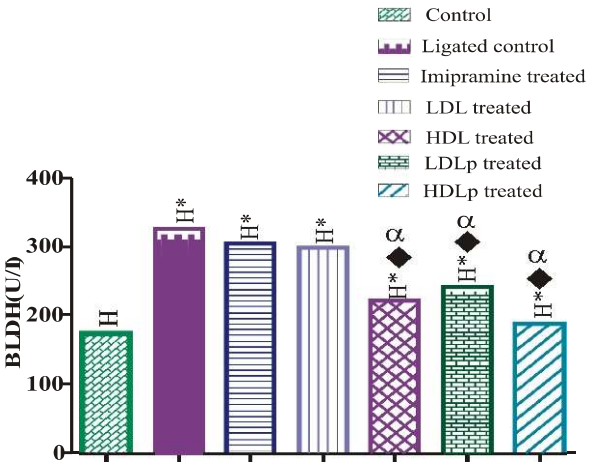

Figure 2: Effect of Licorice on serum lactate dehydrogenase concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, •p<0.05 vs ligated control, [α]p<0.05 vs imipramine; Low dose licorice (LDL); High dose Licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

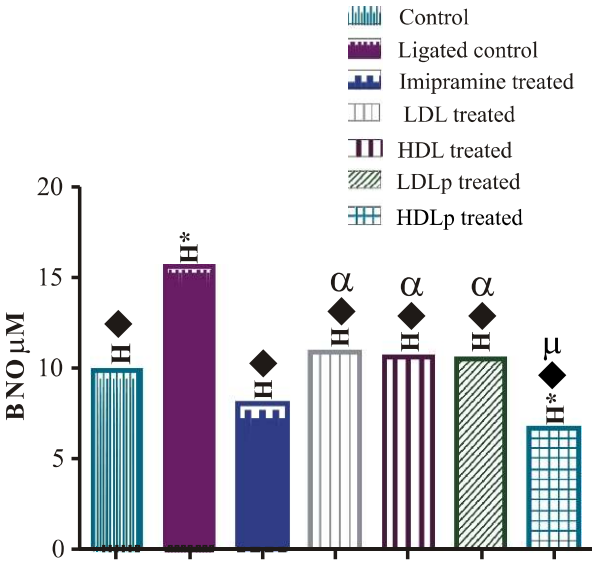

Figure 3: Effect of Licorice on brain nitric oxide concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, [?]p<0.05 vs ligated control, [α]p<0.05 vs imipramine, [μ]p<0.05 vs Low dose licorice (LDL); High dose Licorice (HDL); Low does Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

DISCUSSION

Neuropathic pain can impair quality of life when it is poorly managed. Around 7-8% of adults have pain with neuropathic characteristics and experience a distinct set of symptoms, such as burning and electric-like sensations, and pain resulting from non-painful stimulations (such as light touching), known as hyperalgesia; the symptoms persist and have a tendency to become chronic and respond less to pain medications.[27] Sleep disturbances, anxiety and depression are also

frequent and severe in patients with neuropathic pain.[28]

There are several options for drug treatment as part of an overall approach to improve the quality of life and function of patients' with neuropathic pain, with focus mainly on treating symptoms because the cause of pain can rarelybe treated.[29] In line with that, different classes of drugs with numerous therapeutic recommendations were also proposed for neuropathic pain.[30] Furthermore, pharmacological treatments for chronic neuropathic pain which are effective in <50% of patients may be associated with adverse effects that limit their clinical utility.[31]

Licorice extract constitute rich sources of novel compounds with a variety of pharmacological activities. This study shows that aqueous Licorice root extract increased pain threshold significantly in the licorice treated groups with major effect on the group pre-treated with high dose (150mg/kg) as seen in table 1. Moreover, on day 3 post-surgery, increased pain sensitivity was observed across all groups except ligated control and HDL treated groups. Furthermore, on day 7, HDL, LDLp and HDLp treated groups all demonstrated higher pain threshold compared to animals in the other groups. Again, on day 14 post-surgery, imipramine treated, LDL treated, HDL treated, LDLp and HDLp treated groups all showed appreciable increased pain threshold compared with the normal control group, whereas animals in the licorice pre-treated groups (LDLp and HDLp) demonstrated higher pain threshold compared to the LDL and HDL treated groups and Imipramine treated group.

Increased cortisol concentration is known to be associated with chronic pain which was demonstrated in this study.[32] All the animals demonstrated a significant increase in serum cortisol level where ligated control and imipramine treated groups had the highest concentration, while HDLp treated groups had the lowest. Stressful stimulus coming from acute pain is likely to elicit cortisol secretion and this is known to be commonly associated with hypercortisolism. However, the repeated secretion of cortisol following maladaptive responses to acute pain was reported to perpetuate hypocortisolism, chronic and recurrent pain.[33]

Lactate dehydrogenase (LDH) activity is known to increase in traumatized nerve.[34] Thus, results from this study showed significant increase in LDH concentration in the ligated control, imipramine treated and LDL treated groups when compared with other licorice treated groups. However, reason for increase in brain lactate dehydrogenase concentration may result from increased metabolic demand of the neurons.[35]

Nitric oxide (NO) has been reported to have a pivotal signaling role in both acute and chronic pain at the peripheral and central levels.[36] This present study showed increased BNO concentration in the ligated control group when compared with other groups. This is in consonance with a previous study[37] where increased NO was reported to contribute to various symptoms of chronic pain such as thermal hyperalgesia, mechanical hyperalgesia, and allodynia in sciatic nerve constriction model. HDLp treated groups demonstrated reduced BNO concentration as shown in this present study.

In conclusion, the anti-nociceptive activity of licorice is due to its association with reduced change in cortisol, LDH and BNO serum concentration and the effect is dose and duration dependent, thereby opening a new door in the management of neuropathic pain.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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