Title: Impact of Genotropin on Oxidative Stress, Glutamate, and Nitric Oxide Pathways in a Rat Model of Peripheral Neuropathy

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2024/04/6

Revised date: 2024/11/17

Accepted date: 2024/12/4

Please cite this article as:
Saffarpour, S., Mehraz, A., Sadeghi Tehran, P., Nasirinezhad, F. (In Press). Impact of Genotropin on Oxidative Stress, Glutamate, and Nitric Oxide Pathways in a Rat Model of Peripheral Neuropathy. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2025. Doi: http://dx.doi.org/10.32598/bcn.2025.6536.1
DOI: http://dx.doi.org/10.32598/bcn.2025.6536.1

Abstract

Purpose: While Genotropin, a recombinant human growth hormone (GH), may be a repositioning drug for treating neurological diseases, its effectiveness for neuropathic pain remains uncertain. The current research investigated the pain alleviating effect of Genotropin and its possible effective mechanisms.

Method: Two weeks after chronic constriction injury (CCI) of sciatic nerve, adult male rats were divided into three main experimental groups: Control, Vehicle which received normal saline, and Treatment which received Genotropin (0.3 and 0.6 mg/kg) either alone or in combination with L-arginine, L-NAME, or glutamate (n=8). Pain-related behaviors were assessed using Von Frey filaments, plantar and the Randall-Selitto tests. Blood samples were collected to evaluate relevant oxidant/antioxidant markers.

Results: GH decreased mechanical allodynia ($P<0.05$, $F=2.7$) mechanical ($P<0.01$, $F=3.4$) and thermal hyperalgesia ($P<0.001$, $F=2.5$). Also, pretreatment with 0.3 mg/kg GH abolished the nociceptive effects of L-arginine (500mg/kg) and glutamate (1000nmol) ($P<0.01$; $F=2$, $F=3$), while enhancing the antinociceptive effect of L-NAME ($P< 0.05$, $F=2.8$). It significantly reduced lipid peroxidation ($P < 0.01$, $F =3.7$), restored glutathione, glutathione peroxidase, and superoxide dismutase levels ($P < 0.01$, $F =11$, $F = 10.52$, $F = 5$ respectively) and increased the catalase level ($P < 0.01$, $F =5$) in plasma.

Conclusion: The current data suggests that exogenous GH, alleviates pain and enhance antioxidative factors in the peripheral neuropathic pain model. The glutamate and nitric oxide pathways are also involved in its' antinociceptive effect. It seems that Genotropin can be effective as a repositioning drug in the treatment of neuropathic pain.

Keywords: Growth hormone (Genotropin), Neuropathic pain, Oxidative factors, Nitric oxide, Glutamate.

1. Introduction

Neuropathic pain is a common public health problem caused by a lesion or disease affecting the somatosensory nervous system. Many pathological situations like viral infections (HIV, Herpes simplex, Varicella zoster), metabolic disorders (diabetes), stroke, physical injuries to the central or peripheral nervous system, and toxic effects of drugs can trigger neuropathic pain[1]. The usual signs of neuropathic pain are hyperalgesia and allodynia. Recent data report that half of the patients do not get proper treatment because of the ineffective available analgesics and inadequate data about the pathophysiology of neuropathic pain [2]. Therefore, it is necessary to identify the mechanisms that cause this pain and find new effective or repurposing drugs.
Rising evidence identified that oxidative and nitrosative stress, accompanied by inflammation, constitutes the etiological basis for neuropathic pain[3]. Also in pathological conditions, sustained glutamate release from primary afferent fibers leads to NMDA receptor activation and calcium influx. High intracellular calcium concentration stimulates neuronal nitric oxide synthase and other signaling pathways increasing neuronal excitability, hyperalgesia, and allodynia. L-Arginine, a semi-essential amino acid, is a common precursor for nitric oxide and glutamate in this cellular signalization. L- Arginine, which can be made from citrulline or protein breakdown, is involved in releasing insulin and growth hormone[4-6].

Growth Hormone is well characterized for its essential role in growth and metabolism regulation. Growth hormone is also known for other physiological functions as investigated and confirmed in a variety of assays. Insulin-like growth factor 1 (IGF-1), which its release is stimulated by growth hormone, regulates neural function. Recent data suggested that GH/IGF-1 is concerned with nociceptive modulation [7, 8]. Treatment with GH and GH-releasing hormone ameliorated mechanical and thermal hypersensitivity [9]. Another finding identified the ability of GH treatment to promote axonal regeneration after nerve injury in rats [10]. As recently documented, some neuropathic pain patients have GH abnormalities and GH treatment was effective for fibromyalgia and chronic low back pain syndromes. Also, Ghrelin, a GH releasing factor, has been shown to induce antinociceptive effects in inflammatory and neuropathic pain by regulating the endogenous opioid system, inflammatory factors, and oxidative stress. Also, the involvement of the GH/IGF-1 axis to painful situations such as inflammatory and rheumatic diseases has earlier been described [11, 12].

Although, the above-mentioned studies clearly suggest that GH play a role in pain modulation, the analgesic effect of GH in neuropathic pain related to peripheral injury remains unclear [9]. Since exogenous GH can affect the central nervous system when given peripherally to animals and as a supplemental therapy to humans [13], we hypothesized the identification of new indications and drug repositioning for systemic GH administration. In this research, we investigated the analgesic effect of growth hormone alone and in combination with L-arginine, L-NAME, or glutamate on pain-related behavior in a sciatic nerve injury model of rats and determined the possible involvement of oxidative stress, glutamatergic and nitric oxide pathway in its analgesic effect.

2. Materials and methods

2.1. Experimental animals

Male albino Wistar rats, weighing 200–250 g, were purchased from the laboratory animal breeding center of the Iran University of Medical Sciences and kept under a 12:12 h light: dark

cycle (light on between7:00 a.m. and7:00 p.m.) at 20 °C–25 °C and humidity of 50–60%. Food and water were available ad libitum. All tests were carried out between 10:00 AM and 02:00 PM. Animals were randomly assigned to experimental groups (n = 8 in each group). Caregivers were blended into the grouping. They have been checked on animal health daily and performed regular physical examinations.The experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Ethical Committee of the University (IR.IUMS.REC24501).

2.2. Pain induction

The chronic constriction injury (CCI) model of sciatic nerve, recommended by Bennett and Xie, was used to induce neuropathic pain symptoms [14]. Briefly, the rats were anesthetized with i.p. injection of ketamine (100 mg/kg) and xylazine (15 mg/kg), then, by making an incision in the left thigh parallel to the iliac crest, four loose ligatures were tied around the sciatic nerve before the trifurcation using a 4-0 chromic thread. Sutures only constricted the nerve while its epidural circulation was not interrupted. The experimenter daily checked the surgical site for bleeding, cellulitis, abscesses, and dehiscence.

2.3. Drugs and treatment

Animals were randomly assigned to ten experimental groups (N = 8 per group). The experimental protocol was carried out in two phases:
Study 1: Determining the analgesic effect of different doses of intraperitoneally administration of growth hormone.
Group I: Control (untreated)
Group II: Vehicle (normal saline + CCI)
Groups III and IV: The group received GH (0.3 or 0.6 mg/kg) + CCI
In these groups, the behavioral tests were performed two weeks after CCI surgery and 30 min after intraperitoneal (IP) administrations of GH or normal saline.
Study 2: Investigating the association of nitric oxide and glutamate in inducing analgesic effects of GH
Group V: CCI + L-arginine (500 mg/kg)
Group VI: CCI + L-NAME (20 mg/kg)
Group VII: CCI + Glutamate (1000 nmol)
Group VIII: CCI + GH (0.3 mg/kg) + L-arginine (500 mg/kg)
Group IX: CCI+ GH (0.3 mg/kg) + L-NAME (20 mg/kg)
Group X: CCI + GH (0.3 mg/kg) + Glutamate (1000 nmol)

All drugs, including L-arginine, L-NAME, and Glutamate powders were purchased from Sigma Aldrich (St. Louis, MO, USA) and immediately dissolved in normal saline. Recombinant human GH were received as a gift (ab116162). L-arginine, L-NAME, or Glutamate was injected as a single dose (in a volume of 0.5 ml, i.p.). On the second week after CCI surgery, thirty minutes after GH or saline administration, glutamate, L-arginine, or L-NAME were injected intraperitoneally and behavioral assessments were done 20 min later. The drug selection and the time points were chosen based on other studies [15-17].

2.4. Behavioral assessments

Before actual experimental sessions, animals were handled and habituated to an open Plexiglas chamber for 30 minutes.All behavioral tests were done pre and 14 days following CCI induction.

Mechanical nociception (mechanical allodynia) has been assessed by von Frey filaments (4.56, 4.74, 4.93, 5.07, 5.18, 5.46, and 5.88) based on the method previously described[26]. Each filament was put on the mid-plantar of the hind paw uprightly, by adequate force to cause slight bending against the rat paw, and held there for 3 s. Withdrawal responses were measured by sequentially increasing and decreasing the stimulus strength. The mean withdrawal threshold was assessed by the Dixon nonparametric test[27].

The thermal hyperalgesia determined by the Hargreaves apparatus (Plantar test, 7370, Ugo Basile, Comerio, Italy)[18]. For this purpose, each rat was placed in the plexiglass cage and a thermal beam was applied to the plantar hind paw. Withdrawal latency was automatically recorded from the start of radiation to stop as soon as the rat moved or raised its foot. To avoiding tissue damage, the cutoff was set at 25 sec. Three trials were given at least 1 min apart, and the average values were calculated for statistical analysis.

Reaction to noxious mechanical pressure (mechanical hyperalgesia) has been evaluated via the pressure withdrawal test (Randall-Selitto test)[19]. For this reason, the rats were covered in a towel and an increasing force (48 g/s) was put on the plantar surface of the hind paw until a withdrawal response happened. If the rats did not withdraw, the machine would automatically terminate at 1000 g (25 in-scale units). Two trials were given at least 1 min apart, and the average values were calculated for statistical analysis.

2.5. Biochemical assessment of enzymes related to oxidative stress

The current assay examined the potential effects of intraperitoneal administration of GH on the activity of enzymes associated with oxidative stress in plasma. Immediately, after the completion of behavioral assessments, the animals were deeply anesthetized with mixture of ketamine and xylazin, blood samples were obtained by cardiac acupuncture. The plasma samples were prepared by centrifuging the blood samples at 700– 1000×g at 4 °C for 10 min. Then, the surface plasma was removed by micropipette and kept in the − 80 °C freezer. The activities of enzymes involved oxidative stress, including GSH, GPx, SOD, catalase, and lipid hydroperoxides were measured in plasma samples using the following kits and instructions: GSH/GSSG Ratio Detection Assay Kit (Fluorometric—Green ab138881), Glutathione Peroxidase Assay Kit (Colorimetric ab102530), Superoxide Dismutase Assay Kit (Colorimetric CM706002), Catalase Activity Assay Kit (Colorimetric/Flurometric ab83464), and Lipid hydroperoxide Assay Kit (Colorimetric ab133085).

2.6. Statistical analysis

The Results were addressed as mean ± SEM with $P < 0.05$ calculated as the significance level. Two-way ANOVA and Newman-Keuls tests were used to evaluate significant differences between the groups for the data of behavioral tests. One-way analysis of variance followed by Tukey's post hoc test was used to determine the existence of significant differences in the levels of enzymes involved in oxidative stress between groups. All statistical analyses were calculated by SPSS ver. 20.

3. Results

3.1. Pain behavioral evaluation of different doses of GH

The post hoc analysis determined that CCI resulted in mechanical allodynia two weeks after surgery. Also, at the same time, CCI decreased thermal and mechanical thresholds. Although, the results of behavioral tests showed that single i.p. injection of GH at 0.3 and 0.6 mg/kg alleviated mechanical allodynia as well as thermal and mechanical hyperalgesia in the ipsilateral hind paws of CCI-rats.

A paw withdrawal response to a graded series of von Frey filaments on the animals received 0.3 and 0.6 mg/kg of GH were 6.3 ± 0.5 g and 5.9 ± 0.5 g, respectively, which were significantly ($P< 0.05$, $F=2.7$) higher than the vehicle-treated rats (4.5 ± 0.5 g). These results are presented in figure 1.

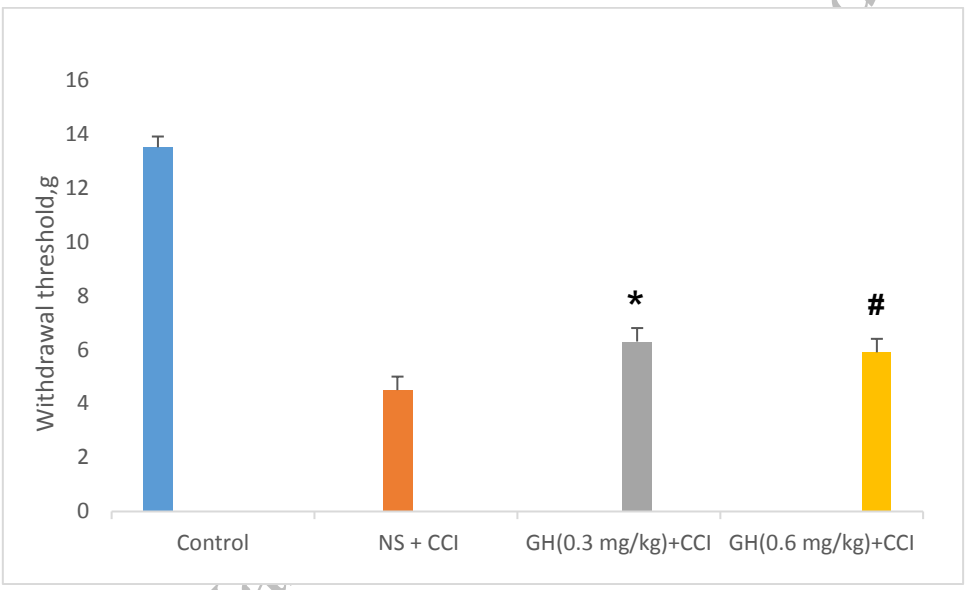


Fig.1.The effect of intraperitoneal administration of different doses of Growth hormone (0.3 and 0.6 mg/kg) on mechanical allodynia (von Frey hairs) two weeks after surgery in CCI rats. GH was administered 30 min before the behavioral test. Values are expressed as mean±SEM (n = 8).* and # $P < 0.05$ compared with the normal saline (NS)-treated group (Vehicle group) and calculated by Two-way ANOVA followed by the Newman-Keuls test.

As Figure (2) presented, the paw withdrawal response to noxious heat (radiant heat test) showed significantly difference between the animals receiving growth hormone (0.3 and 0.6 mg/kg) and normal saline ($P< 0.001$, $F = 2.5$). The mean withdrawal latency in the growth hormone-treated rats were10.37± 0.72 sec (0.3mg/kg) and 11.16 ±0.9 sec (0.6 mg/kg), and the saline treated group was 7.98 ± 0.44 sec.

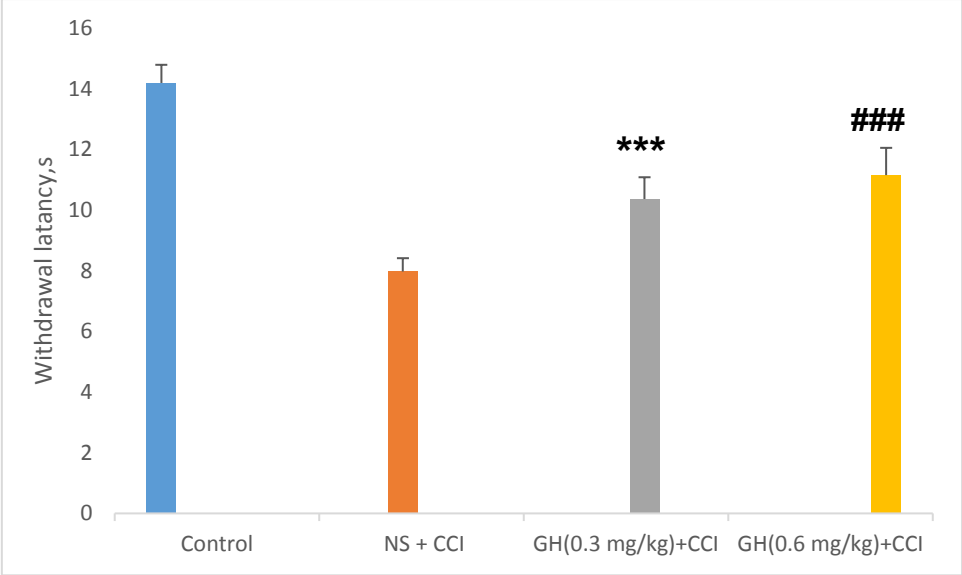


Fig.2.The effect of intraperitoneal administration of different doses of Growth hormone (0.3 and 0.6 mg/kg) on thermal hyperalgesia (radiant heat test) two weeks after surgery in CCI rats. GH was administered 30 min before the behavioral test. Values are expressed as mean ± SEM (n= 8). *** and ### P < 0.001 compared with the normal saline (NS)-treated group(Vehicle) and calculated by Two-way ANOVA followed by the Newman-Keuls test.

The results of assessment the mechanical hyperalgesia (Randall-Selitto test) showed a significant difference (P<0.01, F = 3.4) between growth hormone (0.3 and 0.6 mg/kg) and saline treatment animals. Mean withdrawal threshold values in the animals receiving 0.3 and 0.6 mg/kg of GH were 9.91±0.41g and 10.5± 0.39g, respectively. These were significantly different from animals that received saline (7.85±0.4g) (Figure 3).

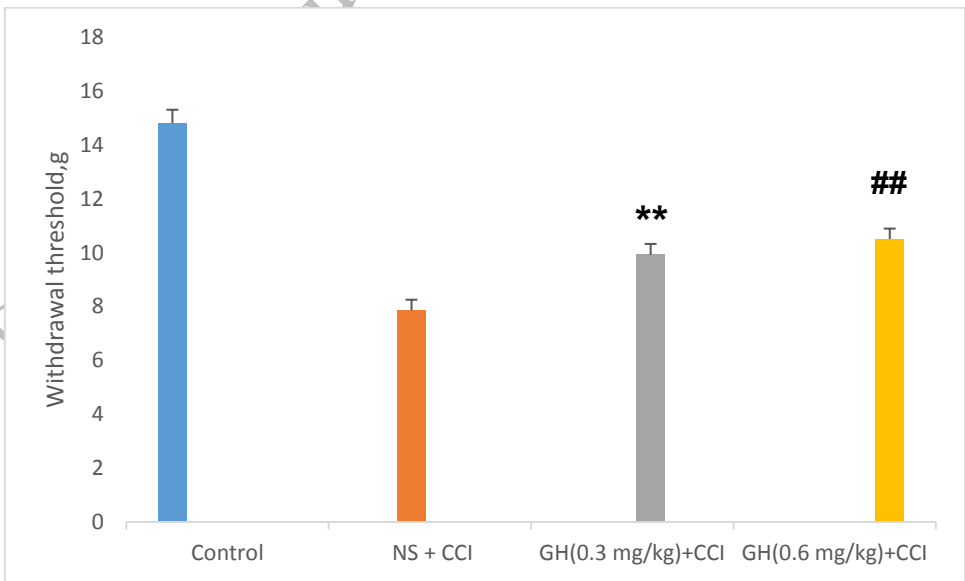


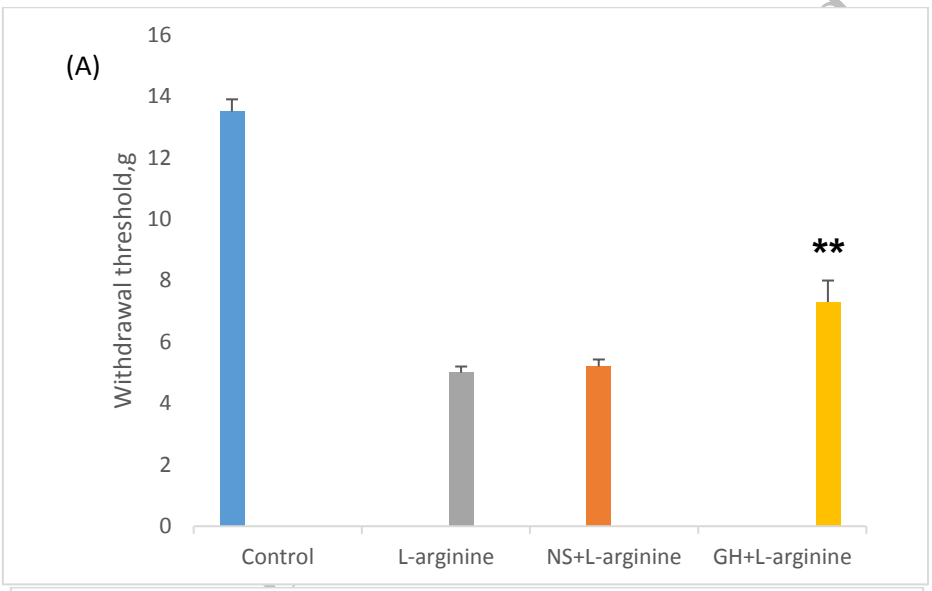
Fig. 3. The effect of intraperitoneal administration of different doses of Growth hormone (0.3 and 0.6 mg/kg) on mechanical hyperalgesia two weeks after surgery in CCI rats.GH was administered 30 min before the behavioral test. Values are expressed as mean ± SEM (n= 8). **and ## P < 0.01 compared with the normal saline (NS)-treated group (Vehicle) and calculated by Two-way ANOVA followed by the Newman-Keuls test.

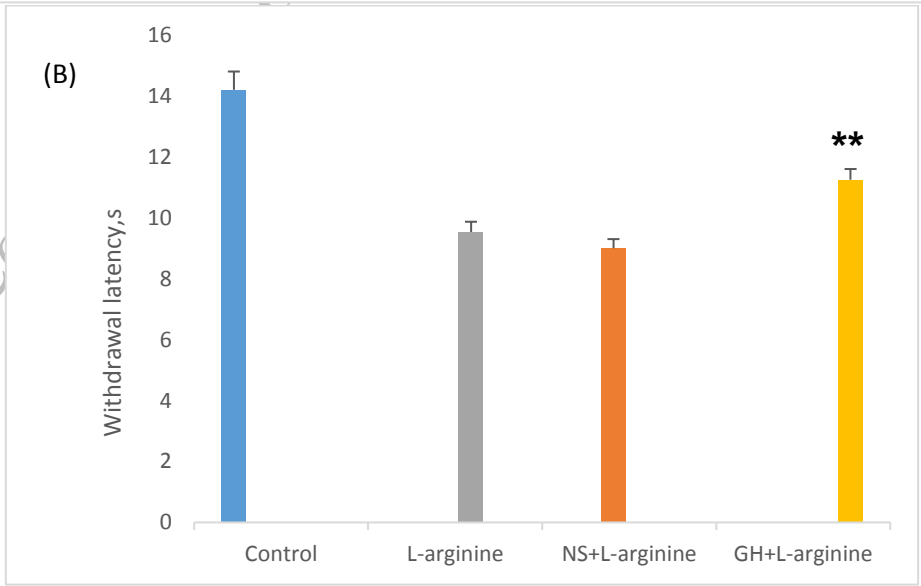
There was no significant differences between animals received different doses of GH in none of the behavioral tests.

3.2. Possible involvement of glutamatergic and nitric oxide pathway in alleviating effect of GH in CCI rats

The analysis pointed out that pretreatment with 0.3 mg/kg GH abolished the nociceptive effects of L-arginine (500mg/kg) (Fig4). In all behavioral tests, a significant difference ($P < 0.01$, $F = 2$) was seen between the GH+ L-arginine and NS±L-arginine groups. There was no significant difference between the mean of NS±L-arginine and L-arginine group.

The withdrawal scores to von Frey filaments, radiant heat, and Randal-Selitto tests were 7.3± 0.7 g, 11.25± 0.35 sec, and 11± 0.45 g respectively in the CCI rats treated with GH and L-arginine. These data were 5±0.2 g, 9.53±0.34 sec and 6.5±0.3 g respectively for the groups treated with saline and L-arginine.





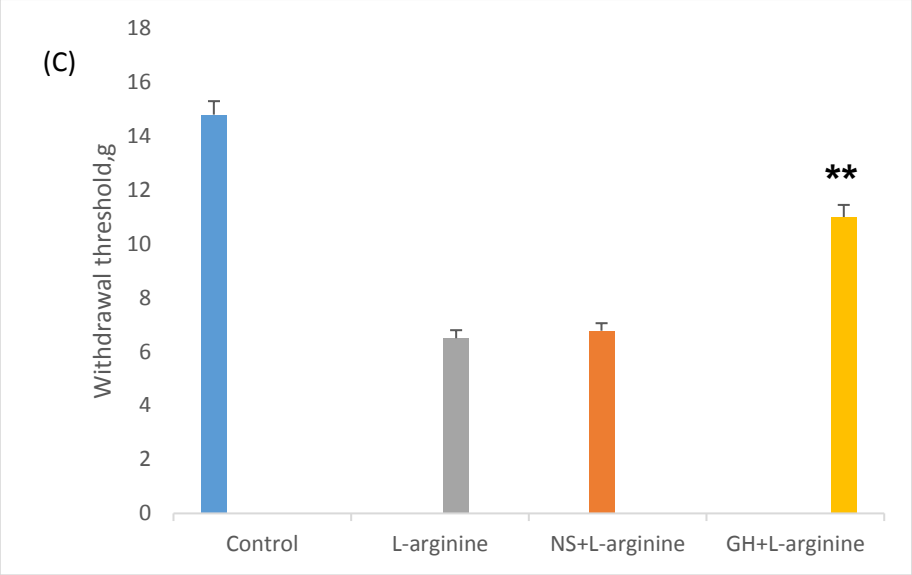


Fig. 4. The effect of pretreatment with GH (0.3 mg/kg i.p.) on mechanical allodynia (A) thermal and mechanical hyperalgesia (B and C respectively) in L-arginine (500 mg/kg,IP) treated groups two weeks after CCI. Values are expressed as mean ± SEM (n =8). ** P < 0.01 compared with the normal saline (NS)-treated group (Vehicle) and calculated by Two-way ANOVA followed by the Newman-Keuls test. All rats underwent CCI except for control.

Figure (5) shows the pretreatment outcome with 0.3 mg/kg of GH on the antinociceptive effect of L-NAME (20mg/kg). The post hoc tests indicated that pretreatment with 0.3 mg/kg of GH enhanced the antinociceptive effect of L-NAME. There was a significant deviation between the GH+ L-NAME and NS±L-NAME treated groups (P < 0.05, F =2.8). There was no significant difference between the mean of NS±L-NAME and L-NAME group. The mean withdrawal latency of von Frey filaments, radiant heat, and Randal-Selitto tests were 12.1± 0.5g, 11± 0.5sec, and11.5 ± 0.58g respectively in the GH+ L-NAME group. Although, these mean response rates were 10.3±0.5g, 8.9± 0.2sec, and 9.8±0.28g respectively in normal saline and L-NAME treated groups.

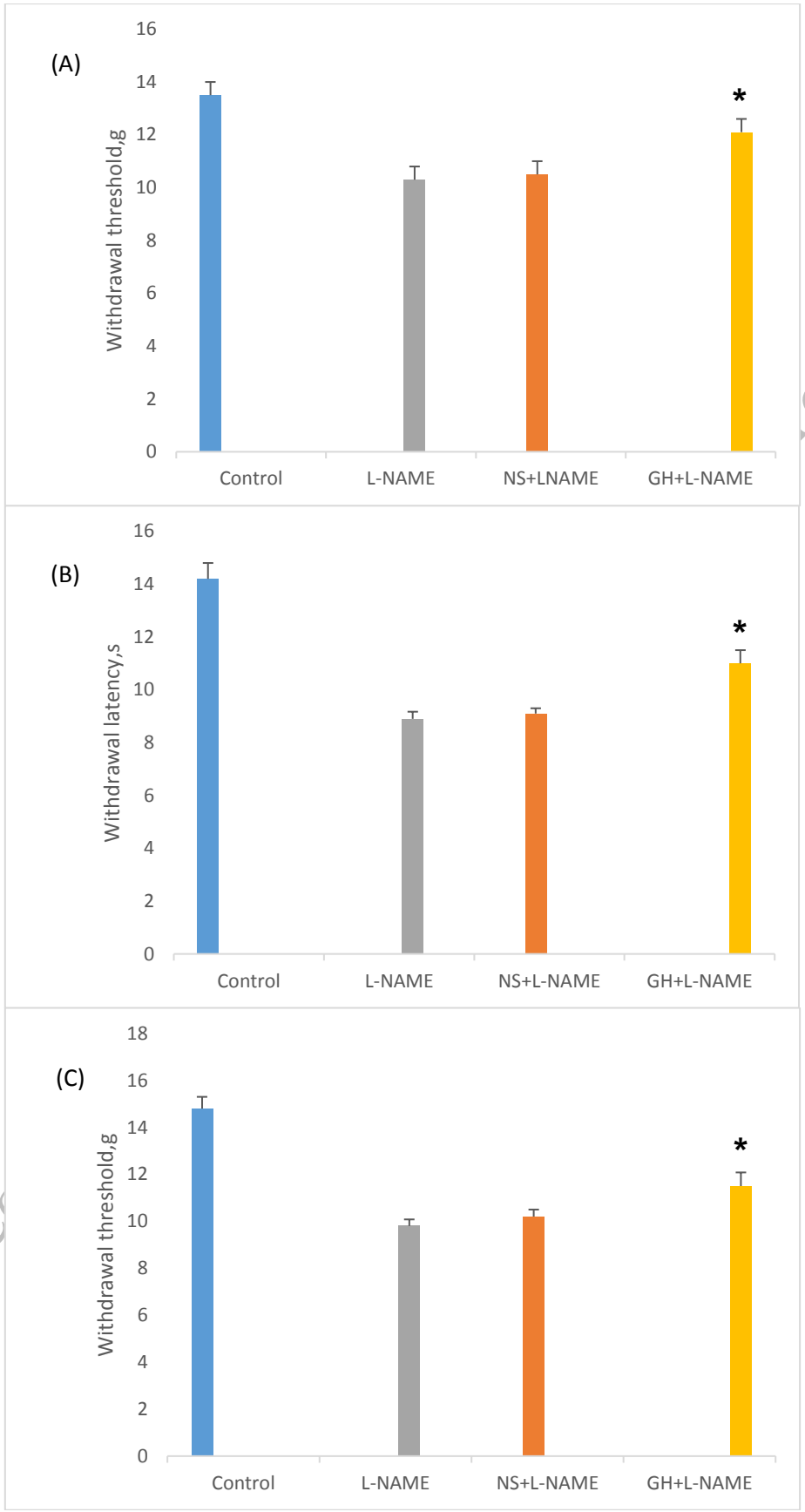
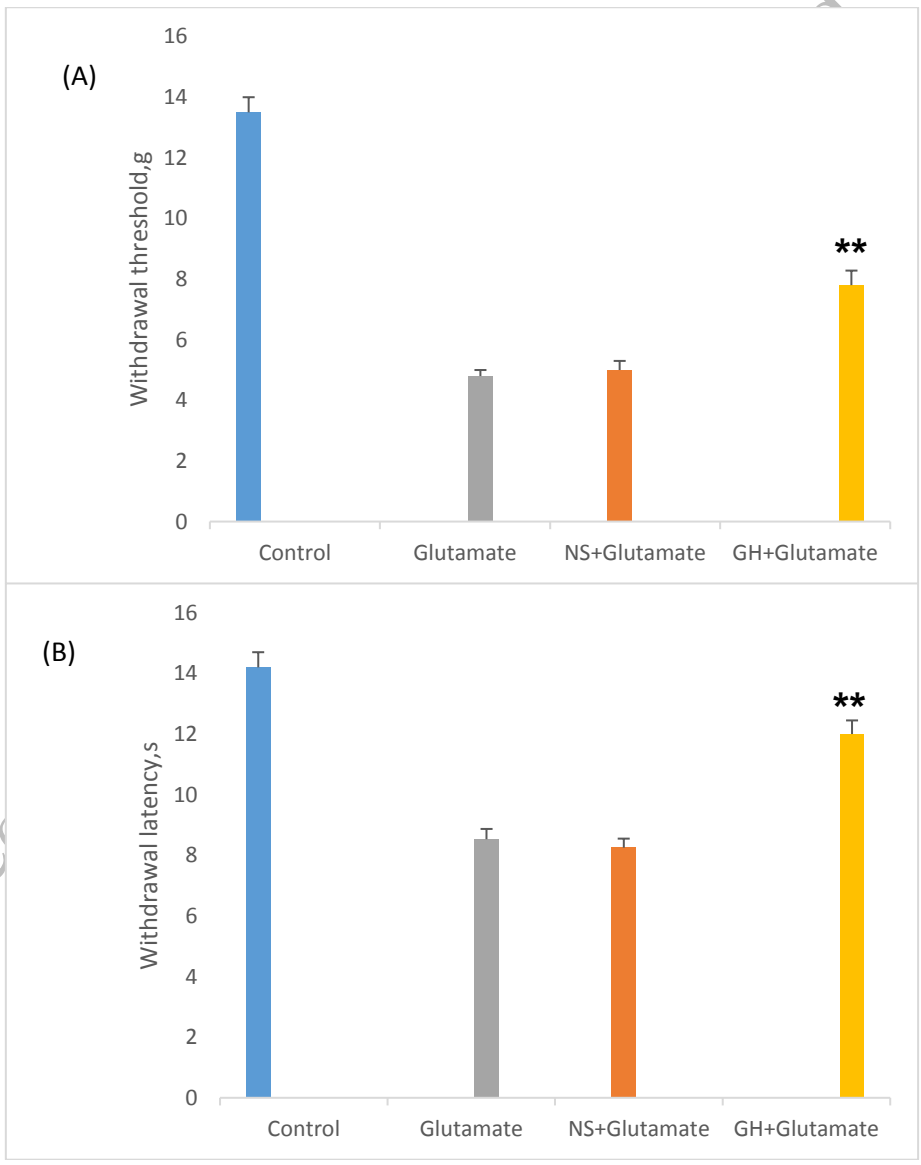

(A)
Withdrawal threshold,g
16
14
12
10
8
6
4
2
0
Control
L-NAME
NS+LNAME
GH+L-NAME
*
(B)
Withdrawal latency,s
16
14
12
10
8
6
4
2
0
Control
L-NAME
NS+L-NAME
GH+L-NAME
*
(C)
Withdrawal threshold,g
18
16
14
12
10
8
6
4
2
0
Control
L-NAME
NS+L-NAME
GH+L-NAME
*

Fig.5. The effect of pretreatment with GH (0.3 mg/kg i.p.) on mechanical allodynia (A), thermal and mechanical hyperalgesia (B and C respectively) in L-NAME (20 mg/kg,IP) treated groups two weeks after CCI. Values are expressed as mean±SEM (n =8). * P < 0.05 compared with the normal saline (NS)-treated group (Vehicle) and calculated by Two-way ANOVA followed by the Newman-Keuls test. All rats underwent CCI except for control group. NS+L-NAME indicates the vehicle group.

Statistical tests indicated that pretreatment with 0.3mg/kg GH significantly (P<0.01, F=3) decreased the nociceptive effects of the glutamate (1000 nmol) (Fig 6). There was no significant difference between the mean of NS±Glutamate and Glutamate group. The withdrawal responses to von Frey filaments, radiant heat, and Randal-Selitto tests were 7.8± 0.48g, 12± 0.45sec, and 10± 0.28g respectively in CCI rats treated with GH,.These data were 4.8 ±0.2g, 8.53 ± 0.34sec, and 6.5 ± 0.3g espectively in the normal saline and glutamate-treated group.



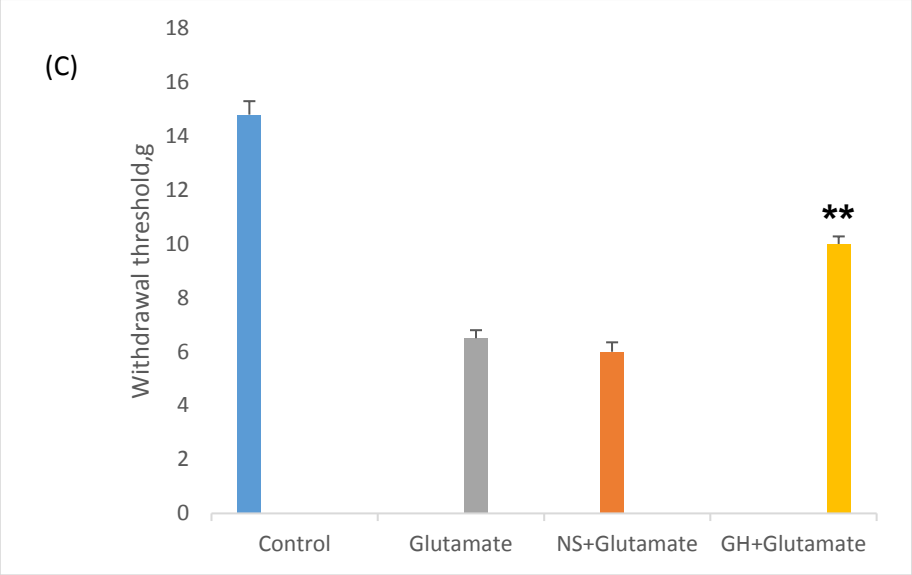


Fig.6. The effect of pretreatment with GH (0.3 mg/kg i.p.) on mechanical allodynia (A), thermal and mechanical hyperalgesia (B and C respectively) in Glutamate (1000 nmol,IP) treated groups two weeks after CCI. Values are expressed as mean ± SEM (n =8).** P < 0.01 compared with the normal saline (NS)- treated group (Vehicle) and calculated by Two-way ANOVA followed by the Newman-Keuls test. All rats underwent CCI except for control. NS+ Glutamate were vehicle group.

3.2. Effects of growth hormone pretreatment on oxidative factors in CCI rats

Chronic constriction injury of the sciatic nerve markedly (P<0.001, F=4) induce oxidative impairment and altered plasma levels of oxidative factors compared with the control group. CCI increased the plasma level of lipid peroxidation. Also, it reduced superoxide dismutase, catalase activity, glutathione, and glutathione peroxidase in plasma. Administration of growth hormone (0.3mg/kg) was noticeably bettered oxidative impairment by the reduction in lipid peroxidation level (P < 0.01, F =3.7), restoration of glutathione, glutathione peroxidase, and superoxide dismutase levels (P < 0.01, F =11, F = 10.52, F = 5) and increasing in catalase activity (P < 0.01, F =5) in plasma as compared with normal saline-treated rats (Table 1).

Table 1 shows that injection of L-NAME (20 mg/kg) with and without growth hormone injection (0.3mg/kg) significantly increased catalase activity (P < 0.01, P < 0.001, F =4.78), and ameliorated the lipid peroxidation level (P < 0.001, P < 0.01, F =3). Also, restored glutathione (P < 0.001, F = 3.2), glutathione peroxidase (P < 0.001, P < 0.01, F =5.2, F = 3), and superoxide dismutase (P < 0.001, F = 4) in plasma compared with normal saline-treated rats. Although injection the L-arginine (500 mg/kg) and glutamate (1000 nmol) has the same consequence on oxidative stress parameters as compared with the normal saline-treated rats; Pretreatment with growth hormone (0.3mg/kg) altered the oxidant outcome of L-arginine and or glutamate (Table 1).

Groups	LPO (nM of MDA/mg)	GPX (µg/mg)	GSH (µg/mg)	SOD (µg/mg)	Catalase (µg/mg)
Control	0.9±0.02	19.2±0.4	27±0.2	50.5±0.25	3±0.2
CCI+NS	2±0.07 ###	11±0.2 ###	20±0.2 ###	35±0.37 ###	11.2±0.3 ###
CCI+GH(0.3mg/kg)	1.7±0.01 **	18±0.34***	24.5±0.25***	45.5±0.3***	24±0.1**
CCI+L-Arginine	2.1±0.08	9.5±0.4	16.8±0.4	39±0.2	0.9±0.3
CCI+GH+L-Arginine	1.8±0.02 **	18±0.4***	26.7±0.01***	46.5±0.5***	27±0.6***
CCI+L-NAME	1.68±0. 03	14.8±0.3	25.8±0.2	46.7±0.3	19.8±0.2
CCI+GH+L-NAME	1.5±0.02 ***	19±0.37***	28.9±0.3***	49.7±0.35***	22.8±0.28***
CCI+Glutamate	1.5 6±0.06	9.8±0.25	18.75±0.32	33±0.4	10±0.35
CCI+GH+Glutamate	1.8±0.02 **	20±0.5***	28±0.3***	47.67±0.35***	30±0.5***

Table 1: Effects of growth hormone (GH) and L-arginine, L-NAME or glutamate on plasma level of oxidative factors in experimental groups. Values are expressed as mean ±SEM (n = 8 in each group). ### P < 0.001 compared to control.**P <0.01 and***P < 0.001 compared to the normal saline-treated groups and calculated by one-way ANOVA followed by Tukey's test.

4. Discussion

The current study indicats that growth hormone treatment alleviates pain-related behavior in CCI model of neuropathic rats suggesting that exogenous growth hormone at a non-toxic dose may be a repositioning drug in neuropathic pain syndrom. Regarding the effective mechanism in the analgesic effect of low dose of growth hormone, its anti-oxidative property can be mentioned. The results of this research showed that in the CCI model, administration of growth hormone can lead to an increase in antioxidant capacity and the ratio of antioxidant to oxidant molecules in blood is improved. In addition, the results have emphasized the involvement of the glutamatergic and the nitric oxide pathway in the analgesic effect of genotropin.

The CCI model reliably induces pain-related symptoms similar to humans in rats, which peak two weeks after surgery and diminish gradually up to week seven . The most common symptoms are hyperalgesia and allodynia, evaluated by behavioral tests [14, 20]. The results of this research also showed that the symptoms of allodynia and hyperalgesia were well established in the animals that underwent CCI.

The effects of GH on growth and metabolism are well known and experimental models have shown that the capillaries of the blood-brain barrier can absorb exogenous GH in animals [18]. However long-term treatment with GH has been associated with side effects such as weight gain, transient fever or hyperglycemia. Therefore, in this assay, we chose acute and low doses of growth hormone that have no side effects based on previous research [9, 15, 16]. Our data showed that systemic administration of either 0.3 mg/kg or 0.6 mg/kg of growth hormone was equally effective in reducing tactile allodynia as well as mechanical and thermal hyperalgesia. These results are consistent with the results of other researches that have shown the antinociceptive effect of exogenous growth hormone in conditions such as fibromyalgia, rheumatic diseases and inflammatory diseases [11, 12, 21]. It has been reported that GH is involved in the process of peripheral sensitization. Although the mechanism involved in

alleviating effect of GH has not been well-investigated, some evidences indicate both GH and its mediator IGF-1 receptors, which are located in neuronal cells, are involved in the pain process and perceptions[22].

It has been reported that IGF-1 increases nociception by activating its receptor, TRPV1,and T-type calcium channel, while GH ameliorates hypersensitivity by suppressing IGF-1 receptors. Dysregulation of IGF-1 signaling results in chronic pain development and antagonists of the IGF-1 receptor and IGF-1 neutralization could improve pain-related behavior [23, 24].Ttreatment with GH could ameliorate mechanical and thermal hypersensitivity by down-regulating IGF receptors in inflamed conditions[13, 25].

Our data showed that administration of growth hormone altered the plasma level of oxidative factors in CCI rats. Chronic constriction injury of sciatic nerve induced the oxidative impairment and altered plasma levels of oxidative factors on the second week after CCI. The plasma level of lipid peroxidation enzymes markedly increased after CCI. While superoxide dismutase, catalase activity, glutathione, and glutathione peroxidase decreased[26].
Systemic administration of Genotropin could increase the antioxidant capacity of plasma[9, 13].
Antioxidant enzymes are capable of stabilizing, or deactivating free radicals such as reactive oxygen species (ROS) before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, Antioxidant enzymes may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. Aerobic life relies on oxidation reactions, but too many reactive oxygen species can be harmful. Fortunately, the body has an antioxidant defense system that can neutralize the harmful effects of ROS. This defense system includes both enzymatic and non-enzymatic mechanisms that work together to eliminate free radicals from the body and prevent oxidation. Antioxidants can be oxidized themselves, which helps to protect the body from damage. In all eukaryotic cells, glutathione is present and is a significant non-enzymatic antioxidant in the body. The major enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione-reductase, catalase, and superoxide reductases. SOD and catalase are the main antioxidant defense against ROS. GPX plays an important role in protecting cells from the harmful effects of peroxide decomposition [8].
In physiological conditions, free oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radicals are made during cellular respiration and scavenged by the intracellular antioxidant defense system. Nitric oxide is a free radical molecule produced from L-arginine in most cells and plays a role in the intracellular defense system. Nitric oxide directly reacts with superoxide and produces peroxy-nitrite. Over-production of free radicals and nitro-oxidative species has a known role in causing oxidative stress. Peripheral nerve injury affects biomolecules, organelles, and antioxidant defense by increasing free radicals, including oxygen and nitrogen reactive species, and causes oxidative stress, mitochondrial dysfunction, activation of microglia, and inflammatory responses. These changes together result in neuropathic pain-related symptoms [26-28].

In this regard, our results showed that treatment with growth hormone reduces the level of lipid peroxidation. It restored glutathione, glutathione peroxidase, and superoxide dismutase levels. Previous studies identified that growth hormone treatment reduced the increased level of oxidative stress and regulated NO production via IGF-I. However, it has also been observed that, in turn, NO stimulates the secretion of GH-releasing hormone and thus increases the secretion of GH [29-31]. Because growth hormone has been reported to modify the L-

arginine/NO pathway, it may be effective in neuropathic pain by scavenging free radicals and nitro-oxidative species.

Growth hormone may also affect glutamate receptors. NMDA receptors (NMDARs) also exist in presynaptic terminals and modulate the evoked and spontaneous transmitter release [32]. From a translational point of view, inhibiting subtypes of NMDARs and/or downstream signaling proteins may provide potential drug targets for chronic pain.

In confirmation of this finding, while we treated CCI rats with L-arginine and or glutamate, they had a lower pain threshold, but pretreatment with growth hormone abolished the nociceptive effect of L-arginine and glutamate. In response to glutamate stimulation, nitric oxide is produced from L-arginine in neurons through an enzymatic reaction. When nitric oxide is produced in glutamatergic neurons, it increases the secretion of prostaglandins, leukotrienes, and substance P; by activating lipoxygenase and cyclooxygenase enzymes, and as a result, sensitizes nociceptors. Over-production of nitric oxide and nitro-oxidative species results in excessive stimulation of glutamate receptors, production of peroxy-nitrite and as a result central sensitivity. Central sensitization plays a role in the development of neuropathic pain [33-35]. So, pretreatment with growth hormone might change the amount of nitric oxide production and has been able to exert its analgesic effects and alleviate the painful effect of L-arginine and glutamate. Other studies have also shown that part of the mechanism of action of some drugs effective in the treatment of neuropathic pain is the inhibition of nitric oxide production, while nitric oxide-releasing drugs and L-arginine cause hyperalgesia and allodynia [35-37].

We observed that systemic administration of L-NAME improved the hyperalgesia and allodynia symptoms in the neuropathic rats. L-NAME is a non-specific nitric oxide synthase inhibitor that can reduce the sensitivity to pain in neuropathic conditions [38-40].Our results indicated that the use of L-NAME with growth hormone increased the analgesic effects of this inhibitor of nitric oxide production. L-NAME may have enhanced the analgesic effect of growth hormone by inhibiting the activity of nitric oxide synthase enzyme and suppressing the production of nitric oxide.

Drug repositioning is a method for identifying new applications or new therapeutic purposes for approved drugs. This method may reduce the duration and cost of drug development by using already approved drugs, which have been tested in human studies for pharmacokinetics and safety profiling. The pathophysiology of neuropathic pain is still poorly understood, making treatment and discovery of drugs a challenging task. Consequently, drug repositioning can be a highly significant technique instead of examining and developing new drug molecules to provide more effective treatment for neuropathic pain with low adverse effects [5-7]. Considering the high prevalence of chronic neuropathic pain and the insufficient effectiveness of available drugs, drug repositioning seems to be a favorable option [48, 49], and the results of current research on the exogenous growth hormone can be promising.

For the first time, this research investigated the effect of growth hormone on the reduction of neuropathic pain and the mechanisms involved in this alleviating effect.

In summary, the results suggests that the exogenous GH could be used as repositioning drug for treatment of neuropathic pain in peripheral neuropathy situation. Administration of growth hormone restored the plasma level of glutathione, glutathione peroxidase, and superoxide dismutase and decreased lipid peroxidation. The glutamate and nitric oxide pathway is probably involved in the analgesic effect of GH and related biochemical alterations. According to these results, it seems that the effect of reducing neuropathic pain can be considered as a new application for growth hormone in non-toxic doses.

Abbreviations
CCI:Chronic Constriction Injury
GH:Growth Hormone
GPX :Glutathione Peroxidase
IGF1: Insulin-like Growth Factor 1
IP:Intraperitoneal
NMDA:N-methyl-d aspartate
NO: Nitric Oxide
NS:Normal Saline
ROS:Reactive Oxygen Species
SOD:Super Oxide Dismutase

Declarations
Ethics approval and consent to participate
The experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP) and were approved by the Ethical Committee of the Iran University of Medical Sciences (IR.IUMS.REC24501)

Availability of data and materials
All the experiment materials were purchased from centers accepted by the Iran University of Medical Sciences, and their quality was guaranteed.
All data presented were original.

Conflict of interest
The authors declare that they have no conflict of interest.

Funding
This experiment received grant from the Physiology Research Center of Iran University of Medical Sciences (Grant number: IR.IUMS.REC24501).

Acknowledgements
This work is granted by the Physiology Research Center of Iran University of Medical Sciences.Dr Fred Nyberg, Uppsala University, Sweden is acknowledged for advisory assistance.

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