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Neuroprotective and Anti-nociceptive Effects of Roflumilast on Vincristine- Induced Neuropathy in Rats

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Abstract

Chemotherapy drugs such as vincristine (VIN) can induce neuropathy, and there is still absence of perfect plan to treat it. The present study was considered to explore effect of roflumilast (Rof.), which is a selective phosphodiesterase 4 (PDE4) inhibitor on VIN-induced neuropathy in rats model. VIN (100 µg/kg, i.p. for 5 days, stop 2 days, and then continued for 5 days) was administered to induce painful neuropathy model in rats. Rof. (3 mg/kg, oral) and gabapentin (60 mg/kg, oral) were administered before 3 days from inj. of VIN and continued for 3 weeks. Numerous parameters were performed; the behavioral tests measurement at different days (0, 7, 14, and 21); rota rod, thermal hyperalgesia (hot plate), and cold allodynia (tail immersion) tests. Measurement of biochemical parameters; measuring; tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) in serum and Nitric oxide (NO) and malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione(GSH) levels in nerve tissue, Furthermore, histopathological changes in sciatic nerves were also studied. Administration of Rof significantly improved the VIN-induced behavioral alterations, biochemical parameters and histopathological changes. Co-administered Rof. Ameliorated VIN-induced painful neuropathy, which might be attributed to neuroprotective and antinociceptive effects by subsequent reduction in oxidative stress and anti-inflammatory actions.

Keywords; Roflumilast, Vincristine, Neuroprotective, Anti-nociceptive, Anti-inflammatory.

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Introduction

A complex condition like neuropathic pain may be developed when nerve fibres are injured or dysfunctional. A wide variety of insults including metabolic disorders like diabetes mellitus, traumatic nerve injury, and neurotoxic drugs like peripheral neuropathy resulted from vincristine related with loss of sensation and numbness of the feet, hands, and legs attended by painful tingling or burning sensation (Visovsky, 2003; Said G., 2007). Hyperexcitability of the nociceptors and variations in central pathways that alter sensory transmission are main characterization of these neuropathies (Salih et al., 2019; Amir R, 2006). Also it is described by the sensory defects like unpleasant abnormal sensation, dysesthesia, an increased response to painful stimuli, hyperalgesia, and pain in response to a stimulus that does not normally provoke pain, allodynia. Peripheral neuropathic pain is commonly perceived in patients with cancer, AIDS, long-standing diabetes, lumbar disc syndrome, and multiple sclerosis (AL-BADRY et al., 2020; Jaggi AS *et al*., 2011).

Chemotherapy induced peripheral neuropathy (CIPN) has a high degree of resemblance in design and spectrum of clinical signs caused by several chemotherapeutic agents (e.g. vincristine, platins, thalidomide), the symmetrical glove and stocking distribution with mainly sensory symptoms. The pathophysiology is poorly assumed but interfering with tubulin function and direct injury to sensory nerve cell bodies of dorsal root ganglia has been found (Hausheer, 2006). The symptom of neuropathic pain management due to chemotherapy mostly depend on the use of antidepressants, anticonvulsants, anaesthetics and opiates. Though there is a perfect medical need for novel treatments to develop safety and efficacy (Dworkin, 2007).

Vincristine is a naturally vinka alkaloid, widely used and potent chemotherapy agent. Vincristine response rate in the first-line treatment in cancers is reach 76 - 92% if treatment is complete. Vincristine is used also to treat a variety of solid mass tumours including small cell lung carcinom and cervical carcinomas (Brown *et al*., 2017). The principal mechanism of vincristine inhibits tumour growth is through binding to and stabilizing

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heterodimer tubulin, causing in interfering with mitotic spindle formation and causing an inhibition of mitosis (Olziersky and Labidi-Galy, 2017).

Roflumilast is a potent and selective inhibitor of phosphodiesterase-4 (PDE4), that indicated for treatment and decrease the risk of chronic obstructive pulmonary disease (COPD) exacerbations, also in patients with severe COPD related with chronic bronchitis (Wedzicha *et al*., 2016b).

Selective inhibition of PDE4 inhibits the hydrolysis of cyclic adenosine monophosphate (cAMP) in inflammatory cells (Contreras *et al*., 2017). Increased intracellular cAMP which results in a wide different processes of anti-inflammatory effects, including; reduced the release of inflammatory mediators in neutrophils, declined the release of cytokines, decreased the expression of cell surface markers in many cell types, and decreased apoptosis (Porpodis *et al*., 2015).

Inflammatory mediators and cytokines suppression frequently explains into benefits for patients with COPD exacerbations with elevated markers of inflammation compared to patients with baseline disease (Mackay *et al.*, 2017). Roflumilast decreases allergen-induced inflammation (Howell *et al.*, 2018) and has been revealed to stabilize lipopolysaccharide induced systemic inflammation (Beute *et al*., 2018).

The most significant PDE isoforms in respiratory diseases are PDE4 and PDE5. PDE4 is additional subdivided into PDE4A, PDE4B, PDE4C and PDE4D which depend on gene encoding. PDE4B inhibition is related with bronchodilator and anti-inflammatory effects, while PDE4D inhibition is linked with emesis due to its prominence in the brain cells (Kawamatawong, 2017a). Therefore, in the present study was designed to investigate the potential neuroprotective and antinociceptive effect of Rof. As a pretreatment on Sensory- Motor induced by vincristine.

Materials and Methods

Drugs and chemicals

Vincristine sulfate was provided by Sigma Pharmaceutical Company (Egypt) and dissolved in normal saline solution to give a stock concentration

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of 1 mg/ml. Rolumilast was purchased from LUPIN-company (India), and gabapentin also was purchased from local market provided by Mylan-company (Italy) and all the other chemicals and solvents used were of maximum analytical assessment**.**

Animals

The present experimental study was designed as an animal (rat) model for neuropathic pain induction. The treatment and follow up period for the animals were continued for 3 weeks. Forty eight albino female rats were kept at constant environmental and nutritional conditions at room temperature with a 12 h/12 hr. light/ dark cycle and divided into six groups each of eight animals.

Experiment protocol

Group I. Control (Normal gp.) were treated with vehicle (normal saline solution) alone.

Group II. (Induction gp.), Vincristine (0.1 mg/kg /day, IP.) was administered for 5 days, and stop 2 days, and then injection of vincristine continued for the next 5 days (BANG *et al.*, 2016).

Group III. (Standard group), animals were pretreated with gabapentin (60 mg/kg /day, oral) before three days from inj. vincristine and continued for 3 weeks (MANGAIARKKARASI, 2015).

Group IV. Animals were pretreated with roflumilast (3 mg/ kg /day, orally) before three days from inj. vincristine and continued for 3 weeks (ZEEMAN, 2016).

Blood samples obtained from rats by cardiac puncture under ether anesthesia and then sacrificed by cervical dislocation. The blood was collected and separated by centrifugation at 3,000 rpm and stored (serum) at -20ºC and used for the various biochemical and enzyme analysis. Sciatic nerves of rats were isolated and preserved in the deep freezer at -80 C until analysis (AL-REJAIE et al., 2015).

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Behavioral assessment

The experimental animals will be subjected for various behavioral studies for assessment of hyperalgesia and allodynia and performed on (0, 7, 14 and 21 days respectively).

1. Rota rod test;

In order to evaluate the motor coordination of the animals, the rats were placed one by one on the rotating rod that was set to 20-25 rpm, 2 trials each rat and scored for their latency to fall in each trial. The falling time of each rat from rotating spindle was recorded during 5 minutes period (Wang *et al.*, 2016).

2. Thermal heat hyperalgesia (Hot plate test):

Thermal heat hyperalgesia of the hind paw was assessed using Eddy's hot plate. The temperature of the plate was maintained 52.5 ± 0.5°C. The rats were placed on the top of a controlled preheated plate to assess the withdrawal response of hind paw to the nociceptive threshold. The cutoff times of 20 seconds was maintained for thermal hyperalgesia (Bhardwaj *et al*., 2016).

3. Cold-allodynia; (tail immersion test),

The tail was immersed in a cold water container by maintaining a constant temperature (0- 4 ^{o}C). Duration of time taken for withdrawal of tail from cold water was noted. A cut-off time of 20 sec was maintained to prevent tissue injury. The procedure was repeated three times for each animal and the mean values are taken in respect (Bhardwaj *et al*., 2016).

Measurement of biochemical parameters;

Preparation of serum samples

Blood samples were aspirated from heart of rats after 21 days, directly by intracardiac puncture by disposable plastic pyrogen free syringes and immediately transferred into plastic test tubes without anticoagulant. The clot was dispersed with

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glass rod and then centrifuged for 15-20 minutes at 3000 rpm and the supernatant was used for the estimation of serum TNF-α and IL-6 levels.

Determination of serum TNF-α and IL-6 levels

Estimation of pro-inflammatory cytokines in serum. Serum levels of TNF-α and IL-6 were estimated by commercially available ELISA kit.

Preparation of tissue homogenate

After the animals have been anesthetized by ether and sacrificed, sciatic nerves were quickly excised, placed in chilled phosphate buffer solution (PH 7.4) at 4 ^{0}C. Specific weighed of nerve tissue was then taken to prepare 10% tissue homogenate using the same buffer solution utilizing tissue homogenizer at 4^{0}C. All preparations were freshly prepared and kept frozen (-70^{0}C) unless worked immediately (Dey *et al*., 2014).

Lipid peroxidation measurement: MDA is a product of lipid peroxidation will be estimated by commercially available ELISA kit.

Determination of antioxidant: SOD and GSH, the concentration of nerve tissue SOD and GSH levels were measured using ELISA technique.

Histopathological studies

Samples of sciatic nerve were stored in fixative solution (10 % formalin) and cut in to 4 μm thickness. Staining was done by using hematoxylin and eosin as described by standard procedure. Nerve sections were analyzed with a light microscope (40x) for axonal degeneration. The slides were coded and examined under light microscope looking for histopathological changes and assessed through longitudinal -section of the sciatic nerve that signed with; demyelination of nerve fibers, degree of nerve tissue disruption, and regularity of axons with vacuoles (Gong, *et al.,* 2016).

Statistical analysis

All the results were expressed as mean ± standard error of means (SEM). The data from the behavioral results were statistically analyzed by two-way analysis of variance followed by bonferroni's post hoc-test. The data from the biochemical results were statistically analysed by one way ANOVA followed by Tukey's multiple range tests. $p \leq 0.05$ was considered to be statistically significant.

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Results

1. Effect of roflumilast on behavioral tests in vincristine-induced Sensory- Motor neuropathy in rats

1.1. Effect of vincristine

Vincristine treated group significantly reduced the falling time latency score in rota rod test (44.2± 0.87), (23.07± 0.53) and (24.28 ± 0.51), when compared to control treated group (78.22± 1.13), (76.46 ± 1.28) and (76.04± 1.09) on 7, 14, and 21 days respectively, fig. (1).

Moreover, vincristine administration significantly decreased the time threshold of thermal hyperalgesia (44.2±0.66), (23.07±0.41) and (25.28 ± 0.44) in comparison with control group (78.22 ±1.23), (76.46±1.09) and (78.04± 1.18) on 7, 14, and 21 days respectively, fig. (2).

Also, vincristine administration was associated with reducing the time threshold of tail withdrawal immersion test (10.59 ±0.09), (9.94 ±0.09) and (9.77± 0.08), when compared to control group (16.95±0.11), (17.09 ± 0.13) and (17.84± 0.1) on 7, 14, and 21 days respectively, fig. (3).

1.2. Effect of roflumilast

Treatment with roflumilast significantly raised the falling time score in rota rod test (63.63± 0.76), (64.63± 0.84) and (66.6± 0.71) in comparison with vincristine group (44.2±0.87), (23.07±0.53) and (24.28± 0.51) on 7, 14, and 21 days respectively, fig. (1).

Treated group roflumilast increased the time threshold of thermal hyperalgesia (59.63 ±0.61), (58.63±0.64) and (62.6±0.66) when compared to vincristine group (44.2±0.66), (23.07±0.41) and (25.28 ±0.44) on 7, 14, and 21 days respectively, fig. (2)

Also, roflumilast treated group significantly enhanced the time threshold of tail withdrawal immersion test (14.11±0.12), (13.45±0.08) and (13.77±0.09) in comparison with vincristine treated group (10.59± 0.09), (9.94±0.09) and (9.77± 0.08), on 7, 14, and 21 days respectively, fig. (3).

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2. Effect of roflumilast on biochemical parameters in vincristine-induced Sensory- Motor neuropathy in rats

2.1. Effect of vincristine

Vincristine treated group was related with significant raise the levels of inflammatory mediators including TNF-α, IL-6 and NO (536.28± 5.56), (141.44 ± 2.5) and (97.688 ± 1.94) in comparison with control treated group (240.07± 3.76), (73.78 ± 1.31) and (42.71 ± 0.19) respectively, table (1).

Vincristine administration was associated with significant increase in oxidative stress marker MDA (4.284 ± 0.186), and decrease in antioxidant agents that including GSH (2.83 ± 0.027) and SOD (0.108 ± 0.003) in the sciatic nerve tissue when compared to control treated group (0.695 ±0.022), (13.04 ± 0.189) and (1.834 ± 0.013) respectively, table (2).

2.2. Effect of roflumilast

In roflumilast treated group, the levels of inflammatory mediators TNF-α, IL-6 and NO (270.18 ± 4.05), (90.13±1.78) and (55.321 ± 0.80) significantly elevated in comparison with control treated group (240.07± 3.76), (73.78 ± 0.31) and (42.71 ± 0.19) respectively.

However, in roflumilast treated group, the levels of inflammatory mediators TNF- α, IL-6 and NO (270.18 ± 4.05), (90.13±1.78) and (55.32 ± 0.80) significantly reduced in comparison with vincristine treated group (536.28 ± 10.56), (141.44 ± 2.50) and (97.68 ± 1.94) respectively.

Also, in roflumilast treated group, the levels of inflammatory mediators TNF-α, IL-6 and NO (270.18 ± 4.05), (90.13±1.78) and (55.32 ± 0.80) significantly raised when compared to gabapentin treated group (251.85 ± 3.23), (76.80 ± 1.70) and (43.899 ± 0.49) respectively, table (1).

Roflumilast treated group was assessed with significant decrease in the oxidative stress marker MDA (1.111 ± 0.024), and increase in antioxidant agents GSH (6.924 ± 0.153) and SOD (0.715 ± 0.082) in the sciatic nerve tissue in comparison with vincristine treated group (4.284 ± 0.186), (2.83 ± 0.027) and (0.108 ± 0.003) respectively.

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While, it administration was revealed with significant decrease in antioxidant agents GSH (6.924 ± 0.153) and SOD (0.715 ± 0.082) in the sciatic nerve tissue when compared to control treated group (13.04 ± 0.189) and (1.834 ± 0.013) respectively.

Although, the antioxidant agents GSH (6.924 ± 0.153) and SOD (0.715 ± 0.082) in sciatic nerve tissue of roflumilast treated group had been showed a significant decline when being compared to gabapentin treated group (12.107± 0.79) and (1.816 ± 0.044) respectively, table (2).

3. Effects of roflumilast on histopathological changes of sciatic nerve in vincristine-induced Sensory- Motor neuropathy in rats

3.1. Effect of vincristine

Vincristine treated group was assessed with marked histopathological changes and evaluated by longitudinal -section of the sciatic nerve signed with; sever demyelinated nerve fibers, marked nerve tissue disruption, and irregular axons with vacuoles when being compared to control group (fig. 4A and 4B).

3.2. Effect of roflumilast

Treated group with roflumilast presented by; moderately demyelinated nerve fibers, mild nerve tissue disruption and regular axons with vacuoles when being compared to vincristine group (fig. 4D).

Discussion

In the present, the results of this study indicated that administration of vincristine produced a significant grade of painful neuropathy in rats, displayed as behavioral (rota rod, thermal hyperalgesia and cold alldynia tests), biochemical (TNF-α, IL-6, NO, MDA, GSH and SOD) and histopathological changes. Peripheral neuropathic pain is frequently observed in patients with cancer (Zimmer *et al*., 2018). The pharmacotherapy for neuropathic pain has had a limited success with little or no response to commonly used pain reducing drugs (Mann and Carr, 2018). Therefore, there is a considerable need to explore novel treatment modalities.

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1. Effect of vincristine

1.1. Effect of vincristine on behavioral parameters

In the present study, the effect of vincristine seemed to reduce the time latency of rota rode, time threshold in thermal hyperalgesia and cold allodynia tests in comparison with control group. Reduction the time latency of rota rode test which may be attributed to impairment of motor coordination that associated with muscle weakness, change of body weight and deterioration in general status (Kagiava *et al.,* 2015). Moreover, the decrement of time threshold in thermal hyperalgesia and cold allodynia tests that reduced by vincristine were thought to occur due to central sensitization as well as the hyper-responsiveness of the degenerated Aδ myelinated and C-unmyelinated fibres (Schappacher *et al.*, 2017).

Mika *et al*. (2013), illustrated that activated spinal microglia and astrocytes had a role in development of vincristine induced hyperalgesia and allodynia. These activated glial cells upregulate and secrete substances such as NO, prostaglandins and proinflammatory cytokines; interleukins and TNF-α, these pronociceptive cytokines, well known to activate the NF-kB pathway are also demonstrated to be critically involved in the development of hyperalgesia and allodynia induced by vincristine (Barzegar-Fallah, 2014).

Park *et al.* (2013), reported that vincristine therapy inhibits the axonal transport of the sciatic nerve in rats, thus the direct effect on peripheral nerves may be a further mechanism of vincristine-induced neuropathic pain. Vincristine also associated with decreasing levels of endomorphin-2 in the spinal cord and DRG (Carozzi, 2015).

Besides, massive intracellular calcium accumulation has been implicated to play a pivotal role in vincristine-induced neuropathic pain (Vashistha *et al.,* 2017). This finding agreed with similar studies (Greeshma *et al.,* 2015; Patil *et al*., 2018).

1.2. Effect of vincristine on biochemical parameters

In this study, the levels of proinflammatory cytokines TNF-α, IL-6 and NO elevated in vincristine treated group significantly in comparison with

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control group, that resulted from activated macrophage inducing effect of vincristine and promoted an elevation in levels of pro-inflammatory cytokines TNF-α, IL-6 in serum, NO and ROS, these events create more nerve damage and the accumulation of these cytokines cause peripheral neuropathy (Rivero-González *et al*., 2017). This result was comparable with other studies (Singh *et al.*, 2018; Nie *et al*., 2017).

In the present study, the NO levels have been shown to be raised in tissue homogenate of sciatic nerve in vincristine group. The high generation of NO enhanced by iNOS expression can lead to tissue injury and do damage to cellular constituents through the generation of reactive nitrogen species, such as peroxynitrite (Xu *et al.*, 2016). This finding was agreeable with study (Bujalska- Zadrożny *et al.*, 2016).

In the current study, the effect of vincristine on oxidative stress parameters demonstrated by elevation of MDA and reduction GSH and SOD levels when being compare to control group. The toxic final product of lipid peroxidation, MDA is a sensitive marker of oxidative stress and is responsible for cytotoxic effects and neuronal death (Zimmermann, 2004). Thus, raised levels of MDA in sciatic nerve of vincristine administered rats are one of the characteristic features of neuropathy that agreeable with other study finding (Gong, 2016).

In the present study, GSH levels decreased in sciatic nerve of vincristine treated rats. The decrease in GSH levels represents increased utilization due to oxidative stress. This finding was agreed with the result obtained by (Tanriverdi *et al.*, 2017).

Also, SOD is a major scavenging enzyme that removes toxic free radicals *in vivo*. It was being reduced in sciatic nerve tissue after induction with vincristine. Reduced activity of SOD in sciatic nerve may result in a number of harmful effects due to the accumulation of free radicals (Bansode *et al*., 2014). This data is similar to study (Kishore *et al.*, 2016).

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1.3. Effect of vincristine on histopathological changes

In the present study, histopathological examination of sciatic nerves in vincristine treated group gave the evidence of neuronal damage with; sever demyelinated nerve fibers, marked nerve tissue disruption, and irregular axons with vacuole in comparison with control group. The axonal degeneration has been described due to these events, suggesting that cellular oxidant and inflammatory mediators play an essential role in the pathogenesis of painful neuropathy under *in vivo* conditions (Gautam and Ramanathan, 2018).

Vincristine caused localized axonal toxicity as a cause of distal axonal degeneration and it is consistent with the predominant axonal damage. It caused the degeneration of myelinated and unmyelinated fibers. The axonal derangement of nerve fibres due to oxidative stress or metabolic derangement seen with the vincristine (Nicolini *et al.*, 2015). This result was in accordance with other studies (Salih et al., 2019; Bansode *et al.*, 2014; Owoeye, 2017).

2. Effect of roflumilast

2.1. Effect of roflumilast on behavioral changes induced by vincristine

In the current study, the effect of roflumilast demonstrated by increasing time latency in rota rod, time threshold of thermal hyperalgesia and cold allodynia tests. Effect of roflumilast on time latency in rota rod test may be mediated by its main mechanism as inhibitor of PDE4 that found to improve motor function in addition to alleviate astrocyte and microglial activation, decline lysosomal pathology, and restore glutamate transporter expression (Aldrich *et al*., 2016).

Effect of roflumilast on thermal hyperalgesia and cold allodynia by increasing time threshold of these tests. This effect may be due to a potent analgesic effect and it delayed the progress of neuropathic pain induced by chemotherapy. So, the modulation of the cAMP levels may be an important mechanism underlying chemotherapy-induced peripheral neuropathic pain; therefore, PDE4 inhibitor may be excellent applicants for treating this type of pain (Kim *et al*., 2015).

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There's no similar study was noticed, but this study finding is agreeable with studies of other member of PDE4 inhibitors revealed with enhancement in behavioral tests (Kim *et al*., 2015; Kim *et al*., 2016 and Johnston *et al*., 2017).

2.2. Effect of roflumilast on biochemical changes induced by vincristine

In the present study, the effect of roflumilast confirmed by decreasing levels of proinflammatory cytokines TNF-α, IL-6 and NO when compared to vincristine treated group. This recognized effect may be through PDE4 inhibitor that increases level of the cAMP predominantly in both nerve and immune tissues, which then activate cAMP-dependent protein kinase A (PKA). Activation of PKA induces the phosphorylation of a number of regulatory proteins including the cAMP -responsive element -binding protein. This activation of PKA can inhibit NF-κB and then decreases the production of inflammatory cytokines; TNF-α and IL6 (Kim *et al.*, 2015).

Roflumilast was shown to reduce the synthesis and release of pro-inflammatory mediators that are essential in asthma, including, cytokines, and TNF-α (Lin *et al.*, 2016). Also, in this study, roflumilast reduced the tissue NO level in comparison with vincristine treated group and this result was compatible with other study that showed an inhibitory effect of PDE4 inhibitors on iNOS and this enzyme was involved in free radical production and oxidative stress. Thus, leading to reduce peroxynitrite level that participate in many normal cellular processes including; ion transport, transcription, neurotransmission, and neuromodulation (Hussien et al., 2020; Kim and Bit-Na-Ri Park, 2011; Chauhan and Chauhan, 2015)

In the present study, the administration of roflumilast caused a lesson in tissue MDA and elevation in GSH and SOD levels in comparison with vincristine treated group. The effect of roflumilast has antioxidant properties reducing MDA level in tissue homogenate of sciatic nerve and this effect may prevent the increase of calcium levels in the macrophages and suppress the production of TNF-α (Mokry *et al*., 2017). This result was similar to other study finding (Uslukaya *et al.*, 2016).

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Moreover, there was an elevation in levels of GSH and SOD in roflumilast treated group that increased by effect on PDE4 inhibitors have been reported to reduce both proinflammatory cytokine levels, molecules involved in free radical production and oxidative stress, such as iNOS and COX-2, as well as immune cell infiltration into the nervous system (Schaal *et al*., 2008, Kim and Bit-Na-Ri Park, 2011). This finding was comparable to other study (Tikoo *et al*., 2014).

2.3. Effect of roflumilast on histopathological changes induced by vincristine

In the present study, roflumilast effects improved the histopathological changes and signed with; moderately demyelinated nerve fibers, mild nerve tissue disruption and regular axons with vacuoles when being compared to vincristine treated group.

PDE4 inhibitor had been reported to decrease the secondary injury, glial scar formation, myelinated axon damage, and oligodendrocyte death after spinal cord injury (Kim *et al*., 2015; Rosenbaum *et al.*, 2016). There are different studies had been revealed the protective effect of roflumilast in different organs like (Rieder *et al*., 2013; Tikoo *et al*., 2014 and Milara *et al.*, 2015).

Conclusion

1- At their applied doses in the present study, roflumilast showed an improvement effects on behavioral, biochemical, and histopthological parameters.

2- However, similar studies on several animal models are essential to obtain a consistent oversight of the effect of roflumilast on vincristine- induced neuropathy in a clinical situation.

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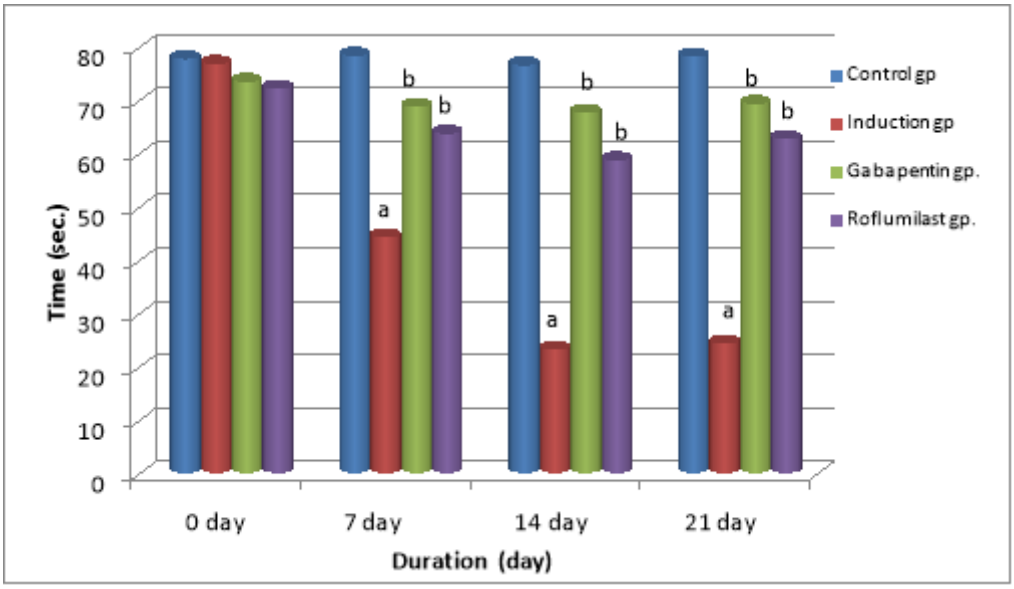

Fig. (1): Effect of roflumilast on rota rod test in vincristine induced Sensory- Motor in rats. a=p ≤ 0.05 in comparison with control group, and b=p ≤ 0.05 in comparison with vincristine group.

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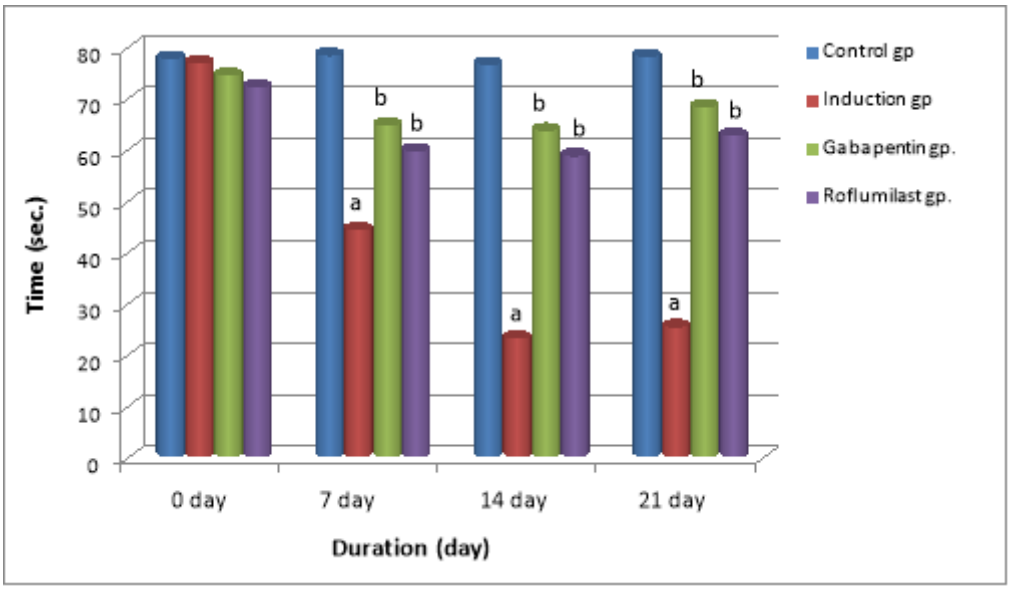

Fig. (2): Effect of roflumilast on thermal hyperalgesia in vincristine induced Sensory-Motor in rats. a=p ≤ 0.05 in comparison with control group, and b=p ≤ 0.05 in comparison with vincristine group.

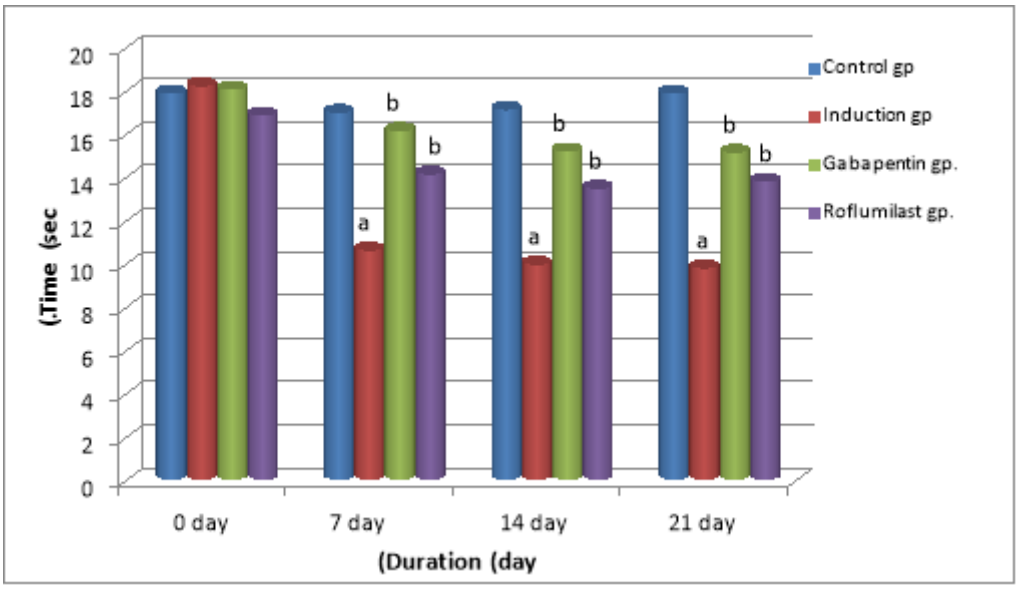

Fig. (3) and associated table: Effect of roflumilast on tail immersion test in vincristine induced Sensory- Motor in rats. a=p ≤ 0.05 in comparison with control group , and b=p ≤ 0.05 in comparison with vincristine group.

Table (1): Effects of roflumilast on TNF- α, IL-6 and NO, levels in vincristine induced Sensory- Motor in rats in 21 days experiment. N= number of animals in each group, SEM= standard error of mean, Vin. = vincristine, a=p ≤ 0.05 in comparison with

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control group, b=p ≤ 0.05 in comparison with vincristine group, and c= p ≤ 0.05 in comparison with gabapentin group.

Groups N= 8	TNF-α (ng/ml) Mean ± SEM	IL-6 (ng/ml) Mean ± SEM	NO (µmol/ml) Mean ± SEM
Control	240.07 ± 0.768	73.78 ± 0.314	42.710 ± 0.197
Vincristine (0.1 mg /kg /day, IP.)	536.28 ± 0.569 [a]	141.44 ± 0.50 [a]	97.688 ± 0.43 [a]
Gabapentin (60 mg/kg /day, oral)	251.85 ± 0.832[b]	76.80 ± 0.705 [b]	43.899 ± 0.29 [b]
Roflomilast (3 mg / kg /day, orally)	270.18 ± 1.05 [abc]	90.13 ± 0.38 [abc]	44.321 ± 0.303 [b]

Table (2): Effect of roflumilast on GSH, SOS, and MDA levels in vincristine induced Sensory- Motor in rats in 21 days experiment. N= number of animals in each group, SEM= standard error of mean, Vin. = vincristine, a=p ≤ 0.05 in comparison with control group, b=p ≤ 0.05 in comparison with vincristine group, c= p ≤ 0.05 in comparison with gabapentin group.

Groups N= 8	GSH (ng/ml) Mean ± SEM	SOD (ng/ml) Mean ± SEM	MDA (nmol/ml) Mean ± SEM
Control	13.04 ± 0.189	1.834 ± 0.0135	0.695 ±0.022
Vincristine (0.1 mg /kg /day, IP.)	2.830 ± 0.027 [a]	0.108 ± 0.0032 [a]	4.284 ±0.186 [a]
Gabapentin (60 mg/kg /day, oral)	12.107 ± 0.492 [b]	1.816 ± 0.0445 [b]	0.754 ± 0.017 [b]
Roflomilast (3 mg / kg /day, oral)	6.924 ± 0.153 [abc]	0.715 ± 0.008 [abc]	1.111 ±0.024 [b]

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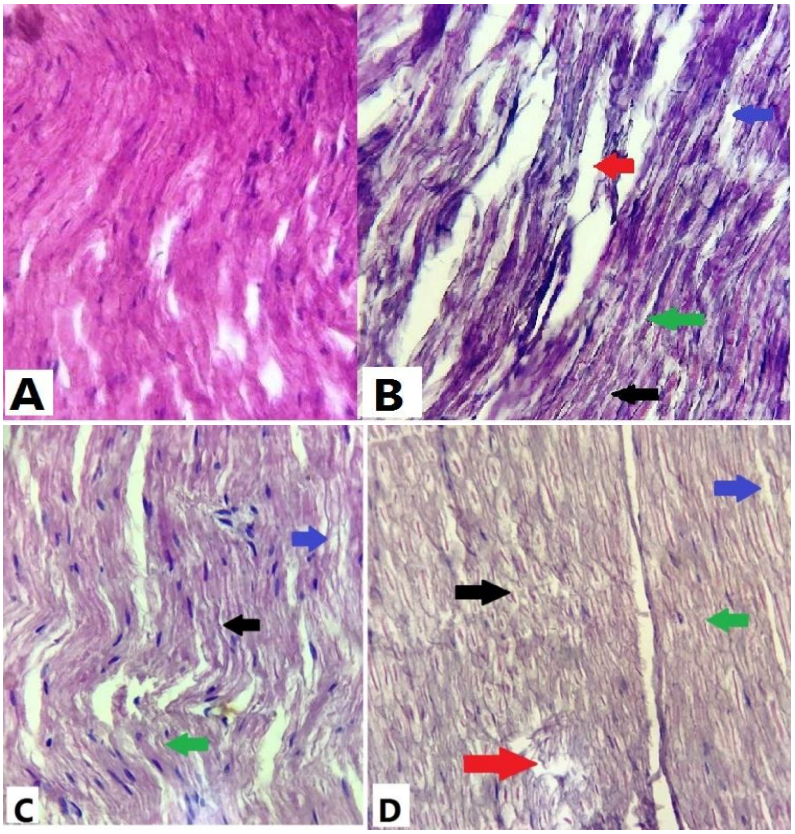

Fig. (4): Light microscope of longitudinal section of sciatic nerve of rats shows; A/ control group; normal sciatic nerve tissue, B/ vincristine treated group shows marked nerve tissue disruption (red arrow), irregular axon (green arrow), sever demyelinated nerve fibers (black arrow), with vacuoles (blue arrow), C/ gabapentin treated group; mild demyelinated nerve fibers (black arrow), mild irregular axon (green arrow), with vacuoles (blue arrow), D/ roflumilast treated group shows; mild nerve tissue disruption (red arrow), moderately demyelinated nerve fibers (black arrow), and regular axon (green arrow), with vacuoles (blue arrow), (H&E, 40X).