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Research Article

PHARMACOLOGICAL IMPACT OF RUTIN AND QUERCETIN IN ALCOHOL-INDUCED NEUROPATHY IN RATS

PRASHANT S KUMBHAR[1], SONALI MANWATKAR[1], BIMLESH KUMAR[1]*, SAMRUDDHI BHIKAJI RAJE[2], AKANKSHA SUNIL CHAVAN[2]

[1]Department of Pharmacology, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India. [2]Department of Pharmacology, Ashokrao Mane College of Pharmacy, Kolhapur, Maharashtra, India.
***Corresponding authors: Bimlesh Kumar; Email: bimlesh1pharm@gmail.com**

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ABSTRACT

Objectives: The purpose of this research study was to assess the effect for the simultaneous administration of quercetin and rutin and their combination in neuropathic pain (NP) caused by alcohol.

Methods: Rats were given ethanol (35% v/v, 10 g/kg, p.o.) for 10 weeks to induce alcoholic NP. Ingested Rutin (100 and 50 mg/kg, p.o.) and Quercetin (40 and 20 mg/kg, p.o.) individually and at their lower doses in combination (50 mg/kg Rutin and 20 mg/kg Quercetin, p.o.) to examine differences in mechanical and thermal hyperalgesia, allodynia, and histopathological parameters. Biochemical measurements of glutathione, thiobarbituric acid reactive species, and protein are used to assess the oxidative stress in alcoholic neuropathy.

Results: The results of the study demonstrated that rats given alcohol for 10 weeks experienced severe mechanical and thermal hyperalgesia, allodynia, and an increased degree of oxidative stress. Lower and greater dosages of both medications alone were used to reduce the symptoms of NP brought on by alcohol. However, simultaneous low-dosage treatment of quercetin and rutin has been proven to significantly improve neurological, histopathological, and metabolic functions when compared to their individual administration. Histopathological studies, which show that nerve regeneration occurs, have provided strong support for the treatment of NP.

Conclusion: Therefore, it is hypothesized that giving quercetin and rutin at the same time may help reduce NP and offer an alternate therapeutic strategy.

Keywords: Rutin, Quercetin, Neuropathic pain, Alcoholic neuropathy, Neuropathy.

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INTRODUCTION

Pain is an undesirable emotional and sensory sensation that typically results from real or possible tissue injury; however, it can also happen when there is no physical impairment [1]. It is a multifaceted experience that combines bodily sensations, emotional reactions, and personal interpretations influenced by social, cultural, and psychological elements [2]. The most troubling sign of peripheral nerve system lesions, which can take many different forms, is chronic Neuropathic pain (NP) [3]. NP is defined as pain that is due to injury or malfunction in either the peripheral (nerves) or central (brain and spinal cord) nervous systems [4]. Features: Frequently described as tingling, burning, firing, or acute are its features [5]. Duration; Furthermore, it may trigger hypersensitivity, in which even slight pressure or touch can be painful [6]. Diabetes, sciatica, phantom limb pain (pain following limb amputation), and postherpetic neuralgia (pain after shingles) are a few examples of NP [7,8]. There are various disorders that can lead to cause severe traumatic injury. These injuries cause damage of various neurons, which can develop the progression of NP, NP caused by mainly somatosensory nerve fibers damage [9,10]. NP has different types of symptoms from common pain and characterized by (I) ALLODYNIA (Painful response to non-painful stimulus), (II) HYPERALSESIA (Increased sensitivity to pain), (III) DYSTHESIA (Unpleasant sensation) and (IV) PARESTHESIA (Abnormal sensation of skin) [11]. The prevalence of NP, which has an enormous effect on the majority of the community worldwide, is estimated to be between 7% and 10% [12]. The reason for this wide range is that different studies have different diagnostic standards, study populations, and techniques. In India's major cities, the incidence of NP is impacted by city lifestyles, healthcare accessibility, and an increase in diabetes patients [13,14]. Despite the absence of significant city-specific NP data, research provides insights into trends in NP incidence across urban populations in India's largest cities. One of the essential features of NP is the presence of pain without an identifiable stimulus. Random pain is caused by ectopic impulses and low-threshold wide myelinated afferents, and VGSC is more responsible for this ectopicity [11,15]. Patients with persistent neuralgia exhibit damage or reduction of sensory axons, which has been positively interpreted with sensory impairment and pain [16], [17]. Therefore, trauma for peripheral nerve results in biochemical and functional alterations at the injured site as well as to further areas of the injured nerve [18,19]. Cytostatic drugs, such as vincristine or Taxol alter a patient's characteristics because they cause axonal trauma or blockade of axonal transport [20]. One significant indicator of nerve damage is the degeneration of Schwann cells. Eventually, it impacts nearby damaged or uninjured neurons by changing the synthesis and production of myelin to growth factors [21]. NP may be caused by a genetic component, channelopathy, hereditary migraine syndrome, and calcium channel mutation. Thus, NP is not caused by a single element. This type of syndrome affects both the sensory and somatic mechanisms [22]. Alcohol is a widely consumed euphoric and addictive drug in society, and its intake causes approximately 3 million deaths globally [23,24]. It also rapidly enters bodily tissues and passes through the blood–brain barrier. It works through impairing with the actions of multiple neurotransmitters, likewise inhibiting the action of glutamate, an excitatory neurotransmitter, as well as increasing the action of γ-amino butyric acid, an inhibitory neurotransmitter [25]. Regular alcohol consumption

can also have negative impacts on the central as well as peripheral nervous systems. Effects caused by excessive alcohol consumption upon peripheral nerves have been investigated using the alcohol-induced NP model [23]. Given the complexity of NP and its several underlying causes, combination therapy is becoming more and more recognized as being crucial to its effective management [8]. A single medication might not adequately treat NP since it frequently involves inflammation, altered neurotransmission, and both peripheral and central sensory impairment. Physicians can target different pathways involved in NP by combining medications with supplementary mechanisms, such as an antidepressant to modulate neurotransmitters and an anticonvulsant to stabilize nerve cells [26]. This can lead to improved pain relief as well as reduced dosages of each drug, minimizing side effects [4]. Furthermore, each person responds differently to NP management, and the type of NP resulting from diseases, such as diabetes, chemotherapy, or nerve damage also ranges significantly. In addition to treating the psychological and physical aspects of NP, drugs can be combined with non-pharmacological methods including as acupuncture, physical therapy, as well as cognitive behavioral therapy to recover patient results also quality of life [24]. NP is a complicated disorder caused by abnormal sensory processing caused on by nervous system damage or malfunction. Despite advances in pharmacotherapy, the clinical challenge of effectively managing NP remains because limited efficacy and adverse effects associated with present treatments [27,28]. Fruits, vegetables, and medicinal herbs have a widespread-ranging diversity of naturally occurring chemicals called flavonoids, which have become of demand for their potential therapeutic effects for the alleviation for NP [29]. This comprehensive investigation focuses at the pre-clinical data about flavonoids' potency for various NP experimental models [20]. The molecular findings behind the analgesic are the advantage of flavonoids, including their neuroprotective, anti-inflammatory as well as antioxidant actions, through a review of the literature [30]. Furthermore, a review of the bioavailability and pharmacokinetic properties of flavonoids, emphasizes their potential and challenges for their therapeutic implementation. By management of several molecular pathways associated with nociceptive processing and neuroinflammation, the investigation highlighted potential outcomes of flavonoids to alleviate NP [31]. The many groups of flavonoids, including flavones, flavanols, flavanones, and flavanols, are investigated to understand the structure-activity relationships and possible synergistic interactions with traditional analgesics management Apples, buckwheat, citrus fruits, and many other plants contain rutin, a naturally occurring flavonoid that has demonstrated potential within the management of NP [32]. The mechanisms by which it minimizes NP are associated through its neuroprotective, anti-inflammatory, as well as antioxidant properties. Because oxidative stress is a significant etiology of nerve injury in NP, rutin's antioxidant property helps reduce its levels in nerve cells [33]. The rutin eliminates free radicals, preventing oxidative damage to nerves. Since its anti-inflammatory properties, it lowers pro-inflammatory agents, such as cytokines (including interleukin-6 [IL]-6 and tumor necrosis factor-alpha [TNF-α]) that are enhanced within NP and contribute to pain and damage nerves. Furthermore, anti-apoptotic characteristics proven by rutin contribute in preventing nerve cell death and promoting systemic nerve health. According to studies, rutin could possibly be able to reduce NP symptoms likewise hyperalgesia (raised pain sensitivity) and allodynia (pain from non-painful stimulant). This suggests it a potentially useful substance for NP management, particularly when used in combined with traditional therapies [34]. A flavonoid exhibiting potent anti-inflammatory, antioxidant, and neuroprotective characteristics that quercetin has shown efficacy to alleviate NP. This is how it's beneficial [35]. Quercetin relieves NP, where damage from oxidation is the primary source of nerve injury, by declining oxidative stress through neutralizing free radicals [36]. Through performing these factors, nerve cells are protected from injury and dysfunction. Pro-inflammatory agent cytokines, which include TNF-α as well as IL-1β, which are frequently increased within NP, are inhibited by quercetin [37]. NP can be alleviated by reducing inflammation, which also lowers nerve irritation and pain sensitivity. Quercetin affects the number of pain-related paths, inclusive of the suppression of nuclear factor kappa B (NF-κB) as well as MAPK signaling, which are engaged in inflammatory and pain responses. This diminishes chronic pain signaling and modifies pain perception. Quercetin's neuroprotective actions delay the progression of nerve injury by regulating and protecting neurons. Nerve degeneration in chronic NP can be prevented or slowed down with this neuroprotection. Research has demonstrated that quercetin can reduce NP symptoms, including hyperalgesia (elevated pain sensitivity) as well as allodynia (pain from light touch), indicating that it may be an effective way to minimize pain-related behaviors in NP. These characteristics make quercetin a promising natural adjunct therapy for NP that targets the basic sources along with signs of NP [38].

Based to an updated literature review on NP and present medications for it, NP is growing rapidly in many different kinds of disease conditions, including diabetes, cancer, alcoholism, renal or hepatic failure, cardiac arrhythmia, psychiatric patients, and geriatric individuals. Low backache can also result from somatosensory nervous system problems, direct nervous system injury, or neuronal damage. The medical condition is unable to completely eliminated by currently available treatments [39,40]. Numerous pathways that follow the pathophysiology of disorders may represent the reason of this. They have their own mechanism and cannot be blocked by the use of only drugs. Research has also shown that some combination treatments may perform better than single therapies. Non-steroidal anti-inflammatory drugs, analgesics, opioids, trichloroacetic acid (TCAs), SNRI, gabapentinoids, and other synthetic or not natural medications that are currently used for nociceptive pain have also had poor success [41]. Due to prevalent adverse effects, such as nausea, headaches, dizziness, and vomiting, a safe and effective solution that may perform through many pathways in conjunction with NP is required. It was shown that natural sources based on bioactive substances, such as flavonoids, were genuinely effective in helping NP due to their significance that they perform their function by blocking various channels linked to NP and reliable credentials. The primary objective of this investigation is to investigate the individual and combination therapeutic actions of quercetin and rutin on alcohol-induced NP model. Hence, the present investigation was intended to evaluate the pharmacological effects of quercetin and rutin within a rat NP model [20,32].

METHODS

Procurement of chemicals and drugs

We received Quercetin (QU, 99) and Rutin (RU, 99) from Yucca Enterprises, WADALA (E), MUMBAI 400 037, INDIA. Himedia Labs provided the thiobarbituric acid (TBA), Loba Chemie provided the ethylenediaminetetraacetic acid and TCA, and Erba Mannheim provided the protein kit. This study was performed by use of pregabalin from Torrent Pharmaceutical Pvt. Ltd. The standard DMSO was collected from the Pharmaceutics Department of Ashokrao Mane College of Pharmacy in Peth-Vadgaon, Kolhapur. All other chemicals as well as biochemical reagents were of the highest quality for analysis.

Procurement of animals for experimentation

Wistar rats weighing (180–270 g) were used and their housing took place at the Central Animal House Facility, Ashokrao Mane College of Pharmacy, Peth- Vadgoan, Maharashtra, India, accompanied for 12 h dark/light environment and an unchanging temperature of 25–2°C.

The Committee for Control and Supervision of Experiments on Animals (CCSEA) granted its approval for this study under protocol number IAEC/AMCP/01/2022–23, and every step of the process was exactly followed as per CCSEA.

Alcohol-induced neuropathy

An established and reproducible lab animal model for pre-clinical medication assessment for pain-induced neuropathy for a long time of alcohol use serves alcohol-induced NP. Hyperalgesia, allodynia, oxidative stress reduction, decreased nerve conduction velocity, cytokine surge,

overproduction of mediators of inflammation and damage to DNA, an imbalance in the OXPHOS pathway, and dysregulated metabolism of energy impair antioxidant as well as electron transfer homeostasis in nerve fibers and axons are established. Rats had been divided into 10 distinct groups containing six rats in every group (n=6), as shown in Table 1. Group I was given distilled water and it was served as the Normal Control. Rutin 100 mg/kg [42] was given to Group II, Quercetin 40 mg/kg [43] to Group III, and ethanol 10 g/kg of 35% (v/v) was given to Group IV as an experimental control (EC). As the standard group, Group V was given Pregabalin (30 mg/kg) plus ethanol, while Groups VI to X were given various doses of drugs with ethanol in accordance with the design. All behavioral assessments were conducted through a blind observer on the 1st, 2nd, 4th, 6th, 8th, and 10th weeks, and dosing was carried out for 10 weeks [44].

Behavioral tests, such as the Rota rod test for motor co-ordination study, the Hot Plate test for paw heat hyperalgesia, the Paw Heat Allodynia test, the Acetone Drop test for Paw Cold Allodynia, the Pinprick test for mechanical hyperalgesia, as well as Tail Cold-Hyperalgesia test (Tail Immersion), were implemented by a blind observer on the 1st, 2nd, 4th, 6th, 8th, and 10th week before surgery. After isolating the sciatic nerve cell, its homogenate was produced by using 0.1 M tris HCl buffer (pH 7.4) on the last day of the experiment. After combining 1 mL of the homogenate supernatant with 2 mL of cold 10% (w/v) TCA to precipitate protein, the mixture was analyzed using a centrifuge for 20 min at 2,000 g. After completion of centrifugation, two milliliters of the supernatant were removed and it stirred for 10 min over a boiling water bath containing an equivalent amount of (w/v) 0.67% TBA until it turned pink. Then analysis was performed to estimate reduced glutathione (GSH), malondialdehyde (MDA), serum total protein within sciatic nerve tissue, also activity of myeloperoxidase in the surrounding sample of muscle tissue [4,44,45].

Behavioral examinations

Motor co-ordination test (rota rod test)

Rats were placed upon the revolving rod and falling duration was investigated from the roller using a 1-min time limit as part of the Rota-rod apparatus, which were used to assess motor coordination test (grip muscle strength) [46].

Paw heat hyperalgesia (hot plate test)

Eddy's hot plate were utilized to assess thermal hyperalgesia in relation with the thermal nociceptive threshold, as previously reported. Before being used, the plate will be pre-heated and kept at 52.5±2.0°C. The nociceptive threshold was evaluated in seconds through monitoring the rats' hind paw licking after they had been put on the hot plate. The 20-s cutoff will be followed [4,47].

Paw heat allodynia test

The sensitivity to non-noxious thermal stimuli was recorded to evaluate the hind paw's heat allodynia utilizing Eddy's hot plate. Allodynia was evaluated by placing rats on over a plate that had been pre-heated to a controlled temperature of 45±0.5°C. Rats were utilized to evaluate the extent of the nociceptive threshold through withdrawing their left hind paw. The 30-s cutoff was maintained [48].

Table 1: Rat groups and doses of drugs for alcohol induced NP

Groups	Treatment	Doses
Animals without ethanol administration		
I	Normal control	____
II	Rutin	100 mg/kg I P
III	Quercetin	40 mg/kg I P
Animals with ethanol administration		
IV	Experimental control/ ethanolic control (EC)	10 g/kg, 35% v/v, bis in die, p.o
V	Pregabalin+ethanol	30 mg/kg
VI	Rutin-L+ethanol	50 mg/kg, 35% v/v bis in die, p.o
VII	Rutin-H+ethanol	100 mg/kg, 35% v/v bis in die, p.o
VIII	Quercetin-L+ethanol	20 mg/kg, 35% v/v bis in die, p.o
IX	Quercetin -H+ethanol	40 mg/kg, 35% v/v bis in die, p.o
X	Rutin L+Quercetin L+ethanol	50 mg/kg (Rutin)+20 mg/kg (Quercetin)+35% v/v bis in die, p.o

Paw cold allodynia test (acetone drop test)

Using an acetone drop test, like reported by Muthuraman *et al.* (2011), the sensitivity of the hind paw toward non-noxious cold chemical stimulus was estimated. One hundred microlitres of Acetone has been sprayed on the rat's hind paw's plantar surface. The withdrawal response was recorded by licking, shaking, or rubbing the hind paw for a maximum duration of 20 s [46,49].

Mechanical hyperalgesia

Following certain modifications, the Kaur *et al.* (2010) were used to assess mechanical hyperalgesia. By using a bent gauge needlepoint at a 90° angle to the syringe, the damaged hind paw was touched. The strength was adjusted thus it doesn't penetrate the skin, but was strong enough to cause a withdrawal reflex reaction within healthy, non-operated rats. With a 20-s maximum cut-off period, the hind paw withdrawal was observed as a sign of the nociceptive threshold [23,46].

Tail cold-hyperalgesia test (tail immersion test)

Cold hyperalgesia was assessed using, with certain modifications to, the test of tail immersion as explained by Kaur *et al.* (2010). In simply, cold water which is kept between 0 and 4°C was used to immerse the tail's terminal end (1 cm). The response time of the tail withdrawal was measured, with a 20-s maximum cut-off period [50].

Biochemical estimations

MDA estimation

After isolating the sciatic nerve, 0.1 M tris HCl buffer (pH 7.4) was used for make its homogenate. To precipitate the protein, 1 mL of the homogenate supernatant was mixed to 2 mL of cool TCA 10% (w/v) (TCA), and the mixture was centrifuged to 20 min at 2,000 g. Following centrifugation, 2 mL of the supernatant were removed and mixed along with an equivalent volume of TBA 0.67% (w/v) within the boiling water bath up to 10 min, or until a pink tint appears. At 532 nm, the supernatant's absorbance was tested with a blank [51].

Reduced GSH estimation

The sciatic nerve homogenate of tissue was undergone a 10-min centrifugation at 3,000 g. 0.4 mL of double distilled water, 0.5 mL of 5, 5-dithio, bis (2-nitrobenzoic acid), and 2 mL of phosphate buffer (pH 8.4) will be mixed to 0.01 mL of this supernatant. Within 15 min of the process completion, the mixture was vortexed, and then absorbance at 412 nm was measured. The ratio of decreased GSH to was represented as µg/mg [52,53].

Serum total protein estimation

The Lowry *et al.* (1951) method was utilized to determine the protein concentration within the sciatic nerve, with bovine serum albumin served as a standard. Then absorbance was measured at 750 nm using spectrophotometry [36,54].

Histopathological examination

The distal sciatic nerve samples were cut into 4 µm thick pieces and preserved within the (10% formalin) as a fixative solution. As explained by the technique, hematoxylin and then eosin was used for staining. Axonal degeneration was qualitatively examined in nerve sections [4,44].

Statistical analysis

Graph Pad Prism 5.01 software was used to analyze the data and express it as mean±standard error of the mean. Two-way analysis of variance (ANOVA) was utilized to analyze behavioral test data, followed by Bonferroni's multiple range test. One-way ANOVA was used to analyze biochemical test data, followed by Dunnett's multiple range test. The significance level was set at 0.05 for the p-value [45,55].

RESULTS

Alcohol-induced NP in experimental animals

Effect of RUT, QUE, and pregabalin on motor coordination

By a period of 10 weeks of treatment, rats in groups I, II, and III did not exhibit any changes in motor coordination whether given the vehicle, Rutin and Quercetin alone. Furthermore, throughout the course of 10 weeks, no remarkable changes in the normal rats' motor coordination were noticed. Rats within the group that was given alcohol (EC, group IV) showed a progressive decline in motor function from day 0 (21.00±0.89 s) to the end of the 10th week (3.83±0.75 s). Groups VI to X treated with Rutin and Quercetin showed a dose-dependent effects and increased time spent on the rotating rod. Meanwhile, groups VII and X that received Rutin H and Rutin L+Quercetin L+E showed statistically substantial attenuation ($p<0.001$). Rats in V (Pregabalin+E) and group X (Rutin L+Quercetin L+E) showed significantly increased performance from the 6th week (15.50±0.89 s), (14.67±0.51 s) to the 10th week (19.33±1.03 s), (18.83±0.75 s), respectively (Fig. 1).

Effect of RUT, QUE, and pregabalin on hot plate test

The administration of ethanol significantly increased the progress of thermal hyperalgesia. Rats within groups VI to X showed a dose-dependent reduction in the nociceptive threshold for thermal hyperalgesia after receiving drug therapy. When rats in groups I, II, and III were given vehicle, Rutin, and Quercetin, their threshold for thermal hyperalgesia did not change throughout the course of the 10th week. At low dosages, the combination of both medications, Rutin + Quercetin+E (group X), significantly ($p<0.001$) counteracted the effects of alcohol-induced hyperalgesia. The paw withdrawal thresholds for the groups administered with Rutin L+E (group VI), Rutin H+E (group VII), Quercetin L+E (group VIII), and Quercetin H+E (group IX) were 10.50±1.04, 12.50±1.04, 9.66±0.81, and 12.17±0.75 s, respectively, at the last day of the 10th week. By the conclusion of the 10th week, the paw withdrawal threshold of rats in groups V and group X treated with Pregabalin+E and Rutin L+Quercetin H+E had significantly decreased ($p<0.001$) by 0.83 and 0.50 s (i.e., the response was 14.33±0.81, 13.50±1.04 s on the 0th day and 13.50±0.83, 13.00±0.89 s by the finish of the 10th week, respectively). Furthermore, it showed that group V (EC) rats were unable to perform picking up their paws (Fig. 2).

Effect of RUT, QUE, and pregabalin on paw heat allodynia test

The rats in group IV (Experimental Control), which were given simply ethanol, reported a consistent and steady reduction within the paw withdrawal threshold rate from 0 day until the study completion. The result of the paw withdrawal threshold was 23.00±0.63 s on day 0, but as the days proceeded through, it finally reduced to 9.00±0.89 s by the end of the 10th week. The alcohol was subsequently shown to produce a considerable ($p<0.001$) rise in allodynia. After at the end of the 10th week,

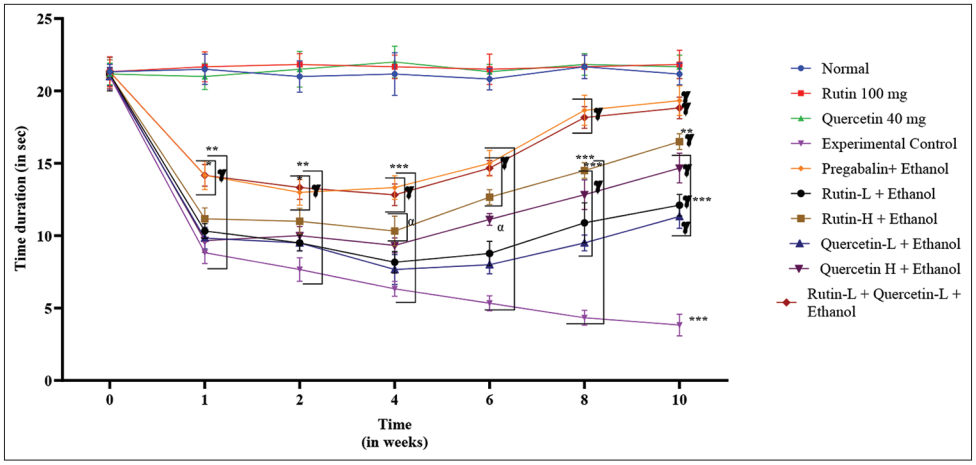

Fig. 1: Effect of drugs treatment on motor coordination. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α ($p<0.001$), β ($p<0.01$), and γ ($p<0.05$) compared with the experimental control group; *($p<0.001$), **($p<0.01$), and *($p<0.05$) in comparison with a normal group**

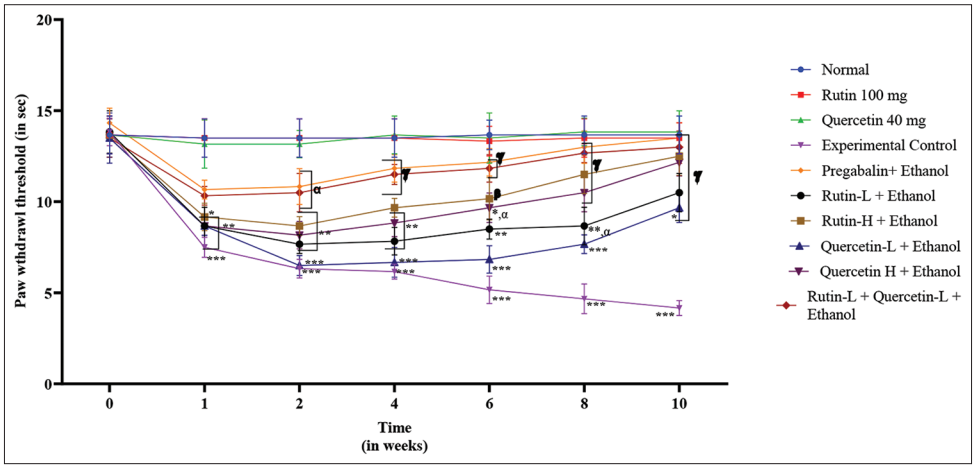

Fig. 2: Effect of drug treatment on hot plate. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α ($p<0.001$), β ($p<0.01$), and γ ($p<0.05$) compared with the experimental control group; *($p<0.001$), **($p<0.01$), and *($p<0.05$) in comparison with a normal group**

groups that received Rutin L+E (group VI, 13.50±0.54 s), Rutin H+E (group VII, 17.00±0.63 s), Quercetin L+E (group VIII, 12.67±0.81 s), Quercetin H+E (group IX, 15.00±0.63 s), as well as Rutin L+Quercetin L+E (group X, 20.67±1.03 s) showed a dose-dependent diminution of alcohol-induced allodynia. Rats within groups I, II, and III were given vehicle, Rutin, and Quercetin did not affect heat allodynia during the same time period. Rats in group V and group X (Pregabalin+E and Rutin L+Quercetin L+E) showed the most noticeable change between weeks 6 (19.33±0.81, 17.67±0.51 s) and 10 (21.00±1.09, 20.67±1.03 s), respectively. Similar findings were detected through various behavioral tests. While it was lower than that of groups V and X (Fig. 3).

Effect of RUT, QUE, and pregabalin on acetone drop test

The significant progression of cold allodynia was caused by alcohol, designated by the rise in paw lifting time caused by the drop of acetone. The treatment of Rutin (50 and 100 mg/kg) and Quercetin (20 and 40 mg/kg) significantly minimized the dose-dependent elevation in paw lifting timing caused by alcohol. The rats in groups V and X (Pregabalin+E and Rutin L+Quercetin L+E) showed the most prominent and positive effects, respectively. In group V and X rats, a significant decline of paw raising reply was noted at the end of the 6th week (2.21±0.02 and 2.37±0.01 s), the 8th week (5.83±0.00 and 1.80±0.01 s), and the 10th week (6.05±0.00 and 1.19±0.00 s), respectively. However, throughout the entire time period, rats within groups I, II, and III that accepted vehicle, Rutin, and Quercetin did not exhibit any changes in cold allodynia (Fig. 4).

Effect of RUT, QUE, and pregabalin on pinprick test

During the pinprick testing, alcohol-induced mechanical hyperalgesia, as shown by a significantly longer hind paw lifting period of time. The administration of Rutin L+E (group VI) (1.42±0.01), Rutin H+E (group VII) (1.08±0.01), Quercetin L (group VIII) (1.48±12), Quercetin H (group IX) (1.35±1.00) and higher doses and a mixture of drugs (group X) (0.86±1.011) reduces the elevation in hind paw lifting time caused by alcohol, that is, mechanical hyperalgesia. Rats in groups I, II, and III did not exhibit any changes in mechanical hyperalgesia during the same interval (10th week) when given vehicle (0.46±0.01), Rutin (0.43±1.21), and Quercetin (0.41±0.12) (Fig. 5).

Effect of RUT, QUE, and pregabalin on tail cold hyperalgesia

However, it showed that treatment with Rutin (50 mg/kg groups VI [10.82±1.32] and 100 mg/kg; group VII [14.25±1.32]), Quercetin (20 mg/kg groups VIII [9.12±1.21] and 40 mg/kg group IX [12.38±1.25]),

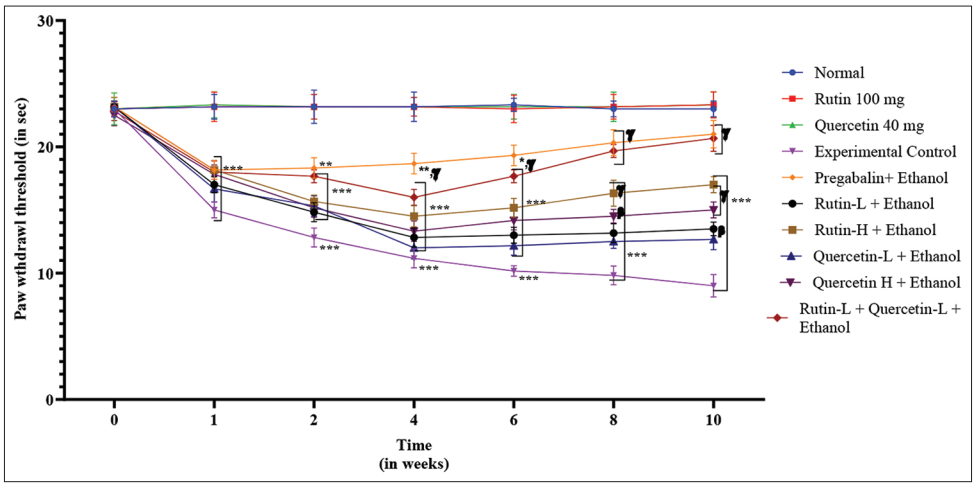

Fig. 3: Effect of drug treatment on paw heat allodynia. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α (p<0.001), β (p<0.01), and γ (p<0.05) compared with the experimental control group; *(p<0.001), **(p<0.01), and *(p<0.05) in comparison with a normal group**

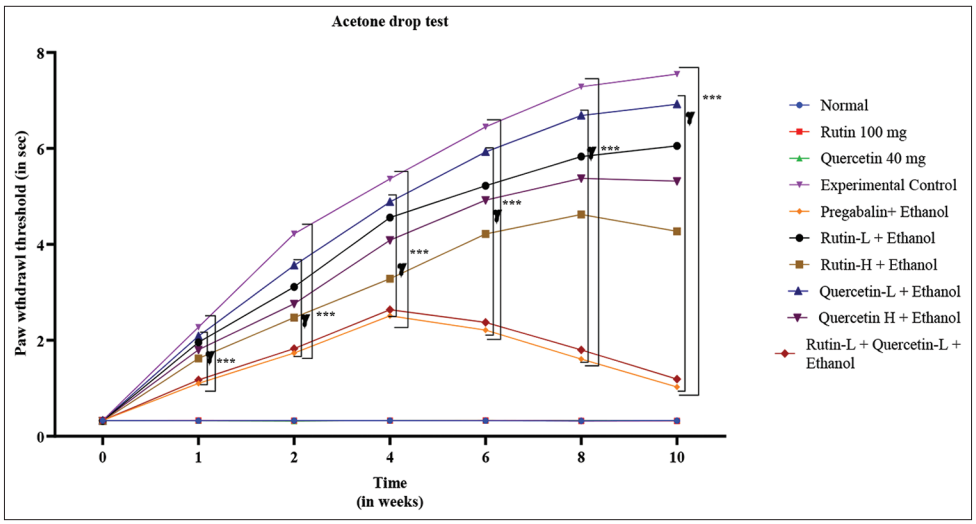

Fig. 4: Effect of drug treatment on paw cold allodynia. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α (p<0.001), β (p<0.01), and γ (p<0.05) compared with the experimental control group; *(p<0.001), **(p<0.01), and *(p<0.05) in comparison with a normal group**

and a both together medication by pregabalin group V (16.24±1.12) and lower doses of both drugs group X (15.95±1.11) attenuated the decrease in withdrawal latency caused by alcohol in reply to noxious stimuli by 10th week. Drugs received by following the pattern of effect on alcohol-induced neuropathy: RUTIN L+QUERCETIN L+E > RUTIN H+E > QUERCETIN H+E > RUTIN L+E > QUERCETIN L+E (Fig. 6).

Effect of treatment upon oxidative biomarkers

While ethanol was administered, the tissue's protein as well as MDA levels elevated also its GSH level dropped indicating an increase in molecular oxidative stress compared with the normal group. It was noticed that using rutin and quercetin at both dosages significantly lowered the biochemical impact of oxidative stress. The co-administration of rutin as well as quercetin at low doses and pregabalin possessed a significantly ($p<0.001$) greater effect upon reducing ethanol-induced oxidative stress than all other treatments. The levels of oxidative stressors were not altered by the alone vehicle, rutin, and quercetin treatment groups. Rats in group X, who received both medications, showed a decrease in protein levels with 4.89±0.13–4.51±0.34 mg/mL, which was comparable with the normal group (group I), which was 4.57±0.24 mg/mL. However, the tissue MDA level of group X rats were 2.28±0.41 nmol/mg of protein, which was much less than that of EC rats ($p<0.001$). Similarly, in rats in group X, the GSH level had been protected to 66.34±0.35 μg/mg of protein (Table 2).

DISCUSSION

Neurotoxicity of the central and peripheral nervous systems, as well as complex neurological defects, are frequently caused by chronic ethanol consumption. Rats' NP caused by alcohol is thought to be the most relevant model because the blood levels of these substances that cause neuropathic changes are equivalent to those in human clinical conditions. One example of the defining symptom is "like tearing the flesh off the body." The present study assessed a number of parameters, including the motor coordination test, mechanical hyperalgesia (pinprick test), paw cold-hyperalgesia (tail immersion test), paw heat-hyperalgesia (hot plate test), and paw cold-allodynia (acetone drop test). Ethanol was effective in this study in inducing neuropathy symptoms in rats. The signs of alcohol-induced NP were alleviated by the use of individual medications, rutin and quercetin at both dosages, as well as pregabalin prescribed as standard. It may be due to the well-established pharmacological effects of rutin, which include acting as an antioxidant, treating diabetic-induced NP, inhibiting mitogen protein kinase, inhibiting the COX-2 pathway, and having anti-inflammatory,

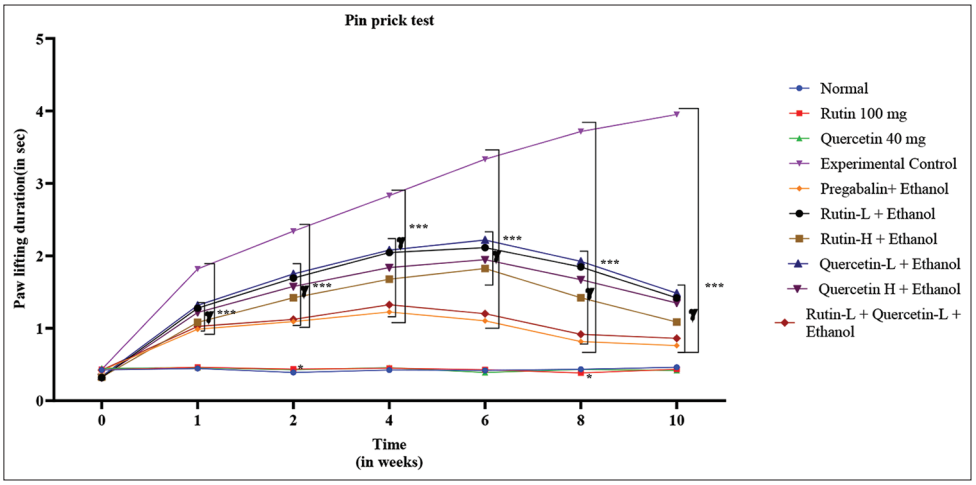

Fig. 5: Effect of drug treatment on pinprick test. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α ($p<0.001$), β ($p<0.01$), and γ ($p<0.05$) compared with the experimental control group; *($p<0.001$), **($p<0.01$), and *($p<0.05$) in comparison with a normal group**

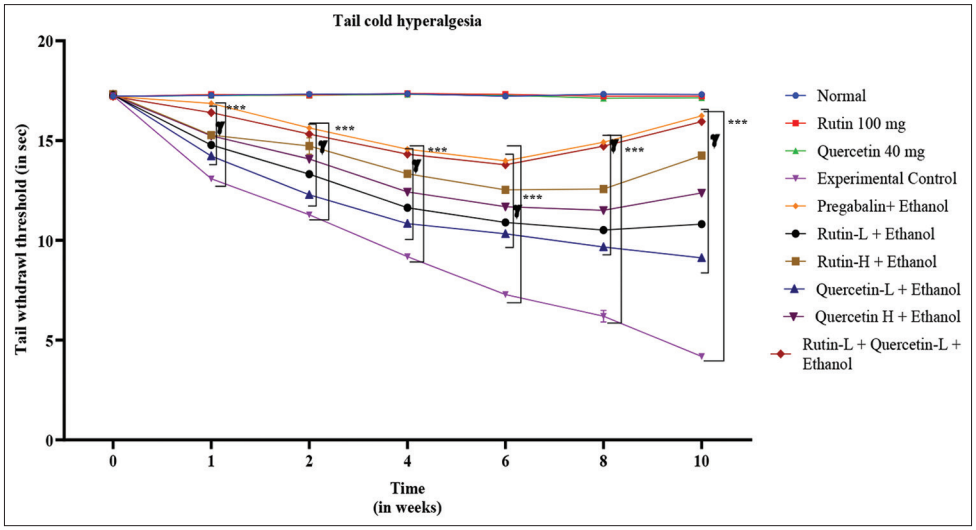

Fig. 6: Effect of drug treatment on tail cold hyperalgesia. Data have been expressed as mean±standard error of the mean (n=6). One-way analysis of variance followed by Bonferroni's test where α ($p<0.001$), β ($p<0.01$), and γ ($p<0.05$) compared with the experimental control group; *($p<0.001$), **($p<0.01$), and * ($p<0.05$) in comparison with a normal group**

Table 2: Effect of rutin and quercetin treatment on oxidative indicators in rats with alcohol-induced NP

Treatment groups	Protein (mg/mL)	MDA (nmol/mg of protein)	GSH (µg/mg of protein)
Normal	4.558±0.00	2.382±0.00	73.08±0.03
Rutin 100 g	4.545±0.00	2.437±0.01	73.10±0.06
Quercetin 40 g	4.568±0.00	2.445±0.01	73.03±0.03
Experimental control	4.868±0.01***	8.872±0.02***	43.35±0.00***
Pregabalin+Ethanol	4.552±0.00γ	2.407±0.01	72.97±0.04γ
Rutin-L+Ethanol	4.727±0.01	6.373±0.06***,γ	57.58±0.01***,γ
Rutin-H+Ethanol	4.597±0.00γ	5.530±0.05***,γ	61.23±0.23***,γ
Quercetin-L+Ethanol	4.670±0.13	6.370±0.09***,γ	48.18±0.01***,γ
Quercetin H+Ethanol	4.570±0.01γ	5.218±0.17***,γ	55.42±0.02***,γ
Rutin-L+Quercetin-L+Ethanol	4.565±0.00γ	2.443±0.02γ	72.15±0.35γ

Data have been expressed as mean±standard error of the mean. (n=6) and analyzed utilizing Dunnett's various ranges test after one-way analysis of variance (ANOVA). α ($p<0.001$), β ($p<0.01$), and γ ($p<0.05$) compared with the experimental control group; *($p<0.001$), **($p<0.01$), and ***($p<0.05$) in comparison with a normal group

anti-rheumatic, anti-cancer, antifungal, and antidepressant effects. Whereas quercetin was found to possess anti-inflammatory, anti-cancer, cardiovascular, neurological, antiviral, hepatoprotective, anti-oxidant, and anti-obesity properties. To alleviate the pain caused by chronic alcohol use, the combination of both of the medications has been decided.

In the present study, every evaluated parameter showed that all treated groups showed attenuation of NP. In alcohol induced NP model found that rats within the group those received a higher dosage of rutin had improved treatment results than the rat's received quercetin. Rutin targets important pathological mechanisms, such as oxidative stress, inflammation, and neuronal dysfunction to produce significant molecular-level effects in reducing NP caused by alcohol. SOD and GPx, both antioxidant enzymes that decrease reactive oxygen species-induced nerve damage, have been elevated as a result of the Nrf2 pathway being activated, boosting the protective effects of antioxidants. Rutin also suppresses the NF-κB signaling pathway, which lowers neuroinflammation through preventing pro-inflammatory cytokines, such as TNF-α, IL-1β as well as IL-6. Furthermore, it restricts the production of advanced glycation end products, which are significant causes of neuronal damage within diabetic neuropathy, and it modifies the polyol pathway. By maintaining membrane potential, lowering cytochrome c release, and lowering the Bax/Bcl-2 ratio and caspase-3 activity, rutin stabilizes mitochondrial function and inhibits apoptosis. Furthermore, rutin reduces neuronal hyperexcitability and pain hypersensitivity by inhibiting voltage-gated sodium channels and TRPV1 channels. Along with these molecular mechanisms make rutin a powerful neuroprotective component for the management of alcoholic NP. Here we also observed that quercetin at their higher doses also alienated the symptoms of NP. In this investigation an interesting finding was observed regarding the potential therapeutic benefit of rutin was better than quercetin. Their combination provided very fast with respect to rats of the group received individual treatment.

In alcohol-induced NP low dose combination of rutin as well as quercetin provided equal results as pregabalin. Because rutin and quercetin have antidepressant and antioxidant properties, co-administration of low doses to rats significantly enhanced the mentioned effect. An earlier study found that co-administration of quercetin and rutin showed notable preventing of obesity is the strategy to inhibit pancreatic lipase, which also inhibits oxidative stress and lowers the formation of pro-inflammatory agent cytokines. Overall, tests of motor coordination, hot plate, paw heat allodynia, acetone drop, pinprick, and tail cold hyperalgesia showed that the combination of low doses of quercetin and rutin, along with pregabalin as a standard, was more effective to their individual doses for attenuating alcohol induced NP. Reduced GSH content in rats fed ethanol and a high-fat diet shows that oxidative stress is likely participated within alcohol-induced neuropathy. Chronic ethanol administration raises the level of MDA (lipid peroxidation product) in the sciatic nerve significantly, which damages tissue by rearranging the double bond in the unsaturated fatty acids and causing lipid membrane destruction. The present study found that groups treated with quercetin and rutin, as well as those receiving pregabalin as standard, had significantly lower MDA and higher GSH. In animals, the antioxidant potential was nearly equal to standard when lower doses of both medications were administered together. In addition, the histopathological evaluation results demonstrate improved sciatic nerve regeneration in drug-treated groups, supporting the attenuation of NP. These studies suggest that *in vivo* administration of both Rutin and Quercetin may have effects resembling to those seen with non-steroidal anti-inflammatory medication therapy, such as diclofenac. As with most non-steroidal anti-inflammatory drugs, that is also proposed that the mechanism of action of Rutin and Quercetin may be linked to the inhibition of cyclooxygenase, which is responsible for the synthesis of prostaglandins. Moreover, when given in combination shows a significant synergistic effect.

CONCLUSION

Hence, it can be concluded that both the Rutin and Quercetin were successfully elicited their pharmacological actions in the treatment of NP. The capacity for both frequent alleviations with attenuation of characteristic symptoms of neuropathy in rats makes this a promising candidate for further development as a treatment for not only in NP but also in neuropathy. Hence, their low-dose combination could be further explored for mechanistic studies on a molecular level and clinical trials to get an alternative formulation for the effective treatment of NP due to different diseases. These findings clearly indicate that NP is caused by various chemicals, diseases, surgeries, injuries, etc., as it leads to damage somatosensory nervous system. Hence, a multifactorial approach for its treatment is also required and our both drugs have given evidence for the same. Their combination can be used for further investigation as an alternative therapy for NP even they can be used to reduce doses and dose frequency of pregabalin.

AUTHORS' CONTRIBUTIONS

Prashant S. Kumbhar: Literature review, Methodology, Data curation, Writing-original draft, and Evaluation; Sonali Manwatkar: Literature review, Bimlesh Kumar: Writing original draft, Review and editing, Supervision, Evaluation, and Visualization; Samruddhi Bhikaji Raje: Writing original draft; Akanksha Sunil Chavan: Writing original draft. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the research.

CONFLICTS OF INTEREST

The authors express no conflicts of interest.

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REFERENCES

1. Gil L, Cheng J. Introduction to acute, chronic, and episodic pain. In: Neuroimmune Interactions in Pain: Mechanisms and Therapeutics.

Vol. 1. Berlin, Germany: Springer Nature; 2023 Jan. doi: 10.1007/978-3-031-29231-6_1
2. Triberti S, Repetto C, Riva G. Psychological factors influencing the effectiveness of virtual reality-based analgesia: A systematic review. Cyberpsychol Behav Soc Netw. 2014 Jun;17(6):335-45. doi: 10.1089/CYBER.2014.0054, PMID: 24892195
3. Kamble S, Poul B, Udapurkar P. Bilayer tablet of tramadol and gabapentin for combination pharmacotherapy of neuropathic pain: Development and characterization. Int J Appl Pharm. 2018;10:100-7.
4. Melkani I, Kumar B, Panchal S, Singh SK, Singh A, Gulati M, *et al*. Comparison of sildenafil, fluoxetine and its co-administration against chronic constriction injury induced neuropathic pain in rats: An influential additive effect. Neurol Res. 2019;41(10):875-82. doi: 10.1080/01616412.2019.1630091, PMID: 31238812
5. Mitsikostas DD, Moka E, Orrillo E, Aurilio C, Vadalouca A, Paladini A, *et al*. Neuropathic pain in neurologic disorders: A narrative review. Cureus. 2022;14(2):e22419. doi: 10.7759/cureus.22419, PMID: 35345699
6. Gilron I, Baron R, Jensen T. Neuropathic pain: Principles of diagnosis and treatment. Mayo Clin Proc. 2015;90(4):532-45. doi: 10.1016/j.mayocp.2015.01.018, PMID: 25841257
7. Jelicic Kadic A, Boric M, Vidak M, Ferhatovic L, Puljak L. Changes in epidermal thickness and cutaneous innervation during maturation in long-term diabetes. J Tissue Viability. 2014;23(1):7-12. doi: 10.1016/j.jtv.2013.11.002, PMID: 24361118
8. Jarvis MF, Boyce-Rustay JM. Neuropathic pain: Models and mechanisms. Curr Pharm Des. 2009;15(15):1711-6. doi: 10.2174/138161209788186272
9. Melkani I, Kumar B, Pandey NK, Baghel DS, Singh S. Statins: A new therapeutic approach for the treatment of neuropathic pain. Int J App Pharm. 2024;16:22-30. doi: 10.22159/ijap.2024v16i5.50456
10. Alshelh Z, Di Pietro F, Youssef AM, Reeves JM, Macey PM, Vickers ER, *et al*. Chronic neuropathic pain: It's about the rhythm. J Neurosci. 2016;36(3):1008-18. doi: 10.1523/JNEUROSCI.2768-15.2016, PMID: 26791228
11. Finnerup NB, Kuner R, Jensen TS. Neuropathic pain: From mechanisms to treatment. Physiol Rev. 2021;101(1):259-301. doi: 10.1152/physrev.00045.2019, PMID: 32584191
12. Flatters SJ, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: Evidence for mitochondrial dysfunction. Pain. 2006;122(3):245-57. doi: 10.1016/j.pain.2006.01.037, PMID: 16530964
13. Tarquini R, Pala L, Brancati S, Vannini G, De Cosmo S, Mazzoccoli G, *et al*. Clinical approach to diabetic cardiomyopathy: A review of human studies. Curr Med Chem. 2018;25:1510-24. doi: 10.2174/0929867324666170705111356
14. Zarei M, Najafi A, Mansouri P, Sadeghi-Yazdankhah S, Saberi H, Moradi M, *et al*. Management of postoperative pain after Lumbar surgery-pregabalin for one day and 14 days-a randomized, triple-blinded, placebo-controlled study. Clin Neurol Neurosurg. 2016;151:37-42. doi: 10.1016/j.clineuro.2016.10.007, PMID: 27764706
15. Baron R. Mechanisms of disease: Neuropathic pain--A clinical perspective. Nat Clin Pract Neurol. 2006;2(2):95-106. doi: 10.1038/ncpneuro0113, PMID: 16932531
16. Attal N, Bouhassira D. Mechanisms of pain in peripheral neuropathy. Acta Neurol Scand Suppl. 1999;173:12-24; discussion 48-52. doi: 10.1111/j.1600-0404.1999.tb07386.x, PMID: 10819088
17. Jaggi AS, Jain V, Singh N. Animal models of neuropathic pain. Fundam Clin Pharmacol. 2011;25(1):1-28. doi: 10.1111/j.1472-8206.2009.00801.x, PMID: 20030738
18. Gracely RH, Lynch SA, Bennett GJ. Painful neuropathy: Altered central processing maintained dynamically by peripheral input. Pain. 1992;51(2):175-94. doi: 10.1016/0304-3959(92)90259-E, PMID: 1484715
19. Knott EP, Assi M, Rao SN, Ghosh M, Pearse DD. Phosphodiesterase inhibitors as a therapeutic approach to neuroprotection and repair. Int J Mol Sci. 2017;18(4):696. doi: 10.3390/ijms18040696, PMID: 28338622
20. Ghate NB, Chaudhuri D, Das A, Panja S, Mandal N. An antioxidant extract of the insectivorous plant *Drosera burmannii* Vahl. alleviates iron-induced oxidative stress and hepatic injury in mice. PLoS One. 2015;10(5):e0128221. doi: 10.1371/journal.pone.0128221, PMID: 26010614
21. De Logu F, Nassini R, Materazzi S, Carvalho Gonçalves M, Nosi D, Rossi Degl'Innocenti D, *et al*. Schwann cell TRPA1 mediates neuroinflammation that sustains macrophage-dependent neuropathic pain in mice. Nat Commun. 2017;8(1):1887. doi: 10.1038/s41467-017-01739-2, PMID: 29192190
22. Melzack R, Coderre TJ, Katz J, Vaccarino AL. Central neuroplasticity and pathological pain. Ann N Y Acad Sci. 2001;933:157-74. doi: 10.1111/j.1749-6632.2001.tb05822.x, PMID: 12000018
23. Kaur H, Kaur M, Singh A, Kumar B. Hepatoprotective potential of aqueous and ethanolic stem-bark extracts of *Pongamia pinnata* against paracetamol induced hepatotoxicity in rats. Int J Pharm Sci Res. 2014;5(10):4275-80. doi: 10.13040/IJPSR.0975-8232.5(10).4275-80
24. Rakhshandeh H, Pourbagher-Shahri AM, Hasanpour M, Iranshahi M, Forouzanfar F. Effects of *Capparis spinosa* extract on the neuropathic pain induced by chronic constriction injury in rats. Metab Brain Dis. 2022 Dec;37(8):2839-52. doi: 10.1007/S11011-022-01094-2, PMID: 36222985
25. Guo D, Hu J. Spinal presynaptic inhibition in pain control. Neuroscience. 2014;283:95-106. doi: 10.1016/j.neuroscience.2014.09.032, PMID: 25255936
26. Jesse CR, Wilhelm EA, Nogueira CW. Depression-like behavior and mechanical allodynia are reduced by bis selenide treatment in mice with chronic constriction injury: A comparison with fluoxetine, amitriptyline, and bupropion. Psychopharmacology (Berl). 2010;212(4):513-22. doi: 10.1007/s00213-010-1977-6, PMID: 20689938
27. Moisset X, Bouhassira D, Couturier J, Alchaar H, Conradi S, Delmotte MH, *et al*. Pharmacological and non-pharmacological treatments for neuropathic pain: Systematic review and French recommendations. Rev Neurol (Paris). 2020;176:325-52.
28. Traa MX, Derry S, Moore RA. Single dose oral fenoprofen for acute postoperative pain in adults. Cochrane Database Syst Rev. 2011;2011(2)(2):CD007556. doi: 10.1002/14651858.CD007556.pub2, PMID: 21328296
29. Cordeiro LM, Soares MV, da Silva AF, Machado ML, Bicca Obetine Baptista F, da Silveira TL *et al*. Neuroprotective effects of rutin on ASH neurons in *Caenorhabditis elegans* model of Huntington's disease. Nutr Neurosci. 2022;25(11):2288-301. doi: 10.1080/1028415X.2021.1956254, PMID: 34311678
30. Hirota T, Fujita Y, Ieiri I. An updated review of pharmacokinetic drug interactions and pharmacogenetics of statins. Expert Opin Drug Metab Toxicol. 2020;16(9):809-22. doi: 10.1080/17425255.2020.1801634, PMID: 32729746
31. Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: Implications for mechanisms of pain. Pain. 2007;131(3):243-57. doi: 10.1016/j.pain.2007.07.026, PMID: 17766042
32. Mousa HA. The potential modulatory effect of rutin on titanium dioxide nanoparticles-induced renal injury in male mice. Int J Pharm Pharm Sci. 2020;12:17-21. doi: 10.22159/ijpps.2020v12i10.39193
33. Xiao WH, Bennett GJ. Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. Pain. 2012;153(3):704-9. doi: 10.1016/j.pain.2011.12.011, PMID: 22244441
34. Moore RA, Barden J. Systematic review of dexketoprofen in acute and chronic pain. BMC Clin Pharmacol. 2008;8:11. doi: 10.1186/1472-6904-8-11, PMID: 18976451
35. Mohamed NZ, Mantawy M, Abd-Alla HI, Aly HF, Ibrahim N, Hassan SA. CCl4-induced hepatonephrotoxicity: Protective effect of nutraceuticals on inflammatory factors and antioxidative status in rat. J Appl Pharm Sci. 2014;4(2):87-100. doi: 10.7324/JAPS.2014.40215
36. Eid HH, Labib RM, Hamid NS, Hamed MA, Ross SA. Hepatoprotective and antioxidant polyphenols from a standardized methanolic extract of the leaves of *Liquidambar styraciflua* L. Bull Fac Pharm Cairo Univ. 2015;53(2):117-27. doi: 10.1016/j.bfopcu.2015.05.002
37. Ye G, Lin C, Zhang Y, Ma Z, Chen Y, Kong L, *et al*. Quercetin alleviates neuropathic pain in the rat cci model by mediating AMPK/MAPK pathway. J Pain Res. 2021;14:1289-301. doi: 10.2147/JPR.S298727, PMID: 34040433
38. Bardestani A, Ebrahimpour S, Esmaeili A, Esmaeili A. Quercetin attenuates neurotoxicity induced by iron oxide nanoparticles. J Nanobiotechnology. 2021 Dec;19(1):327. doi: 10.1186/S12951-021-01059-0, PMID: 34663344
39. Rungratanawanich W, Qu Y, Wang X, Essa MM, Song BJ. Advanced glycation end products (AGEs) and other adducts in aging-related diseases and alcohol-mediated tissue injury. Exp Mol Med. 2021;53(2):168-88. doi: 10.1038/s12276-021-00561-7, PMID: 33568752
40. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr. 2011;3(1):17. doi: 10.1186/1758-5996-3-17, PMID: 21812977
41. Khajuria K, Gupta S, Dogra DR, Kumar D, Khajuria V. Comparison

of pregabalin and nortriptyline on efficacy and safety in postherpetic neuralgia. Asian J Pharm Clin Res. 2021;14(7):74-6. doi: 10.22159/ajpcr.2021.v14i7.41872

42. Ziaee A, Zamansoltani F, Nassiri-Asl M, Abbasi E. Effects of rutin on lipid profile in hypercholesterolaemic rats. Basic Clin Pharmacol Toxicol. 2009;104(3):253-8. doi: 10.1111/j.1742-7843.2008.00368.x, PMID: 19175365
43. Dong YS, Wang JL, Feng DY, Qin HZ, Wen H, Yin ZM, *et al*. Protective effect of quercetin against oxidative stress and brain edema in an experimental rat model of subarachnoid hemorrhage. Int J Med Sci. 2014;11(3):282-90. doi: 10.7150/ijms.7634, PMID: 24516353
44. Kaur M, Singh A, Kumar B, Singh SK, Bhatia A, Gulati M, *et al*. Protective effect of co-administration of curcumin and sildenafil in alcohol induced neuropathy in rats. Eur J Pharmacol. 2017 Aug;805:58-66. doi: 10.1016/j.ejphar.2017.03.012, PMID: 28315678
45. Pottabathini R, Kumar A, Bhatnagar A, Garg S, Ekavali E. Ameliorative potential of pioglitazone and ceftriaxone alone and in combination in rat model of neuropathic pain: Targeting PPARγ and GLT-1 pathways. Pharmacol Rep. 2016;68(1):85-94. doi: 10.1016/j.pharep.2015.06.010, PMID: 26721358
46. Muthuraman A, Singh N. Attenuating effect of *Acorus calamus* extract in chronic constriction injury induced neuropathic pain in rats: An evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC Complement Altern Med. 2011;11:24. doi: 10.1186/1472-6882-11-24, PMID: 21426568
47. Muthuraman A, Singh N. Attenuating effect of hydroalcoholic extract of Acorus calamus in vincristine-induced painful neuropathy in rats. J Nat Med. 2011;65(3-4):480-7. doi: 10.1007/s11418-011-0525-y, PMID: 21404093
48. Khangura RK, Bali A, Kaur G, Singh N, Jaggi AS. Neuropathic pain attenuating effects of perampanel in an experimental model of chronic constriction injury in rats. Biomed Pharmacother. 2017;94:557-63. doi: 10.1016/j.biopha.2017.07.137, PMID: 28780471
49. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of *Albizia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol. 2000;71(1-2):65-75. doi: 10.1016/S0378-8741(99)00192-0, PMID: 10904147
50. Dawane JS, Pandit VA, Bhosale MS, Khatavkar PS. Evaluation of effect of Nishamalaki on STZ and HFHF diet induced diabetic neuropathy in Wistar rats. J Clin Diagn Res. 2016;10(10):FF01-5. doi: 10.7860/JCDR/2016/21011.8752, PMID: 27891351
51. Bilski J, Mazur-Bialy A, Wojcik D, Magierowski M, Surmiak M, Kwiecien S, *et al*. Effect of forced physical activity on the severity of experimental colitis in normal weight and obese mice. Involvement of oxidative stress and proinflammatory biomarkers. Nutrients. 2019;11(5):1127. doi: 10.3390/nu11051127, PMID: 31117199
52. Al Moundhri MS, Al-Salam S, Al Mahrouqee A, Beegam S, Ali BH. The effect of curcumin on oxaliplatin and cisplatin neurotoxicity in rats: Some behavioral, biochemical, and histopathological studies. J Med Toxicol. 2013;9(1):25-33. doi: 10.1007/s13181-012-0239-x, PMID: 22648527
53. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70-7.
54. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193(1):265-75. doi: 10.1016/s0021-9258(19)52451-6, PMID: 14907713
55. Kumar A, Kaur H, Singh A. Neuropathic pain models caused by damage to central or peripheral nervous system. Pharmacol Rep. 2018;70(2):206-16. doi: 10.1016/j.pharep.2017.09.009, PMID: 29475003