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Betula utilis (bark extract) speeds up sciatic nerve function restoration following a compression injury in a mouse model

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Abstract: **Background**: People who experience various traumas often develop peripheral nerve injuries (PNIs). Currently available strategies are insufficient to fully recover nerve damage. Plants are used to treat a variety of illnesses. **Objectives**: In this study, we investigated the possible effect of *Betula utilis* on the restoration of muscle function following an injury to a peripheral nerve. **Methods**: Here, we treated PNI by using bark extract from *Betula utilis*. First, 32 healthy albino mice were equally divided into 4 groups. Bark extract (orally) at varying concentrations (25, 50, 100, and 200 mg/kg) was assessed for two weeks. Behavioral assessment and histology of vital organs were performed for the dose adjustment. Afterwards, for sciatic nerve injury, 32 mice were equally divided into four groups: Control and sham (groups 1 and 2, treated with normal saline) and treatment groups (groups 3 and 4, *B. utilis* extract, 25 and 50mg/kg). Behavioral analyses assess sensorimotor recovery. All observations were analyzed statistically at a p-value of $p<0.05$. **Results**: Behavioral analyses depict an improvement (statistically significant) in the treated group as compared to the non-treated group. Serum samples analysed for oxidative stress markers showed statistically significant ($p<0.05$) results for TAC (Total antioxidant capacity) and TOC (Total oxidative stress) in the treated groups. **Conclusion**: In summary, *B. utilis* extract improved sensorimotor function restoration, and to determine its appropriateness for people, more research is necessary.

Keywords: *Betula utilis*; Grip strength; Peripheral nerve injury; Sciatic functional index

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INTRODUCTION

Traumatic nerve injuries occur due to different issues, ranging from accidents, violence, surgeries, trauma, and falls (Modrak *et al*., 2020). It interrupts the normal functioning of sensory and motor neurons by causing demyelination. These injuries, if left untreated, can cause lifelong disabilities. Despite advances in neuroscience and surgery, the likelihood of improvement for patients undergoing surgical reconstruction of nerve injuries remains low (Pan *et al*., 2020).

PNIs fall among the most critical health issues due to their higher prevalence. These PNIs adversely affect the brain's communication with the target muscles or organs (Hussain *et al*., 2022). It is essential to diagnose and treat PNIs as soon as possible to prevent complications, permanent damage to muscles, and lifelong disabilities in the affected individual. Several factors make it challenging to treat such injuries, including location, intensity, and type of nerve injury. Future treatments for PNIs aim to exploit various approaches to enhance recovery of motor and sensory function after nerve injury (Juckett *et al*., 2022).

Upon nerve damage, a cascade of pathological events initiates phagocytosis, oxidative stress, inflammation, and demyelination at the specific site of nerve degeneration, leading to loss of sensory and motor functions. Mild to moderate nerve injuries can regenerate over time, but recovery is very slow. Therefore, the reinnervation time of the target tissue or muscles takes months or even years. Ultimately, denervated muscles start to shrink, thus leading to dystrophy. Although a variety of therapeutic choices are available, achieving a fully functional recovery remains a significant challenge (Sonawane *et al*., 2023).

Plant-derived bioactive compounds are readily available in nature and offer multiple health benefits. They have anti-inflammatory, antioxidant, analgesic, antimicrobial, and various other medicinal properties. These compounds have the fewest side effects and are a good alternative to synthetic medicines (Marrelli, 2021). In modern research, scientists are working on unexplored bioactive molecules to treat various diseases. Among them, many phytochemicals are neuroprotective for brain diseases (Shoaib *et al*., 2023).

B. utilis is widely distributed in the Himalayan regions, and the bark of this plant contains triterpenoids such as betulin, betulinic acid, and lupeol, which are accountable for antioxidant, antiproliferative (Biswasroy *et al*., 2023), anti-inflammatory, antimicrobial, and anticancerous properties. Moreover, they also play a role in Alzheimer's disease as a cognitive enhancer. Primary human keratinocytes, the triterpene in birch bark, showed promising results in the scratch assay (Ishfaq *et al*., 2023). In India, *B. utilis* bark and roots are used to treat burns, leprosy, bronchitis, jaundice, ear complaints, and as an antiseptic, as well as for wound healing (Kumar Vishwakarma *et al*., 2022). *B. utilis*

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bark is effective in the management and treatment of various medical ailments. Therefore, it is a promising source that can be incorporated in different dosage forms to treat many ailments (Khanam *et al*., 2023a). However, to the best of our knowledge, the bark extract of this plant has yet to be explored for the treatment of nerve injuries. Literature reveals that various components in bark extract possess anti-inflammatory and antioxidant properties necessary for neuroprotection and neuroregeneration. Thus, it provides a clue for its possible use as a neurodegenerative agent.

MATERIALS AND METHODS

Experimental animals housing

The Institutional Animal Ethical Committee of Government College University in Faisalabad gave its approval for the use of healthy albino mice. Mice that weighed between 18 and 30 grams and were 8 to 10 weeks old on average were employed. To prevent interaction, each animal was kept in a single cage. They were maintained in a 12-hour light-dark cycle. For a week, the mice were kept in the animal home with regular feed, ad libitum water and temperatures between 23 and 27°C, with relative humidity between 40 and 60%. Every experiment and behavioral observation was carried out during the light cycle.

Plant collection and processing

The bark of the *B. utilis* plant was collected from Gilgit-Baltistan and subsequently identified by the Department of Botany, Government College University Faisalabad (298-bot-21). Dried *B. utilis* bark was cleaned and then chopped into small pieces before soaking in ethanol. For eight days, *B. utilis* bark was immersed in ethanol. After eight days, it was filtered through filter paper. A rotary evaporator was used to get the powder extract of this plant after evaporating ethanol. Later on, the extract was incubated for a few days until it was thoroughly dried and was stored in an airtight container until further use.

Selection of a suitable dose

Here, albino mice weighing between 20 and 30 grams were divided into treatment and control groups. For two weeks, the treatment group was given varying oral doses of *B. utilis* extract (25, 50, 100 and 200 mg/kg) dispersed in 0.5% carboxymethyl cellulose (CMC). In contrast, the control group was given a daily oral dose of normal saline. To choose an appropriate dosage for additional research, behavioral evaluation and mortality rates were calculated for each group during this time. At the conclusion of the experiment, every animal was sacrificed. Samples were sectioned and stained with hematoxylin and eosin after all important organs were submerged in 10% neutral buffer formalin. Lastly, slides were seen at 4x and 10x magnification using a microscope (AccuScope 3000, USA) fitted with a 5-megapixel camera.

Procedure for nerve injury induction

Following a week of acclimation, all mice had surgery to cause mechanical harm. They received intraperitoneal injections of xylazine and ketamine (70 mg/kg and 5 mg/kg body weight) to induce anesthesia. The mid-thigh area of the right leg was exposed for nerve crush. A fine incision exposed the sciatic nerve. A single investigator then used forceps to press the nerve for 15 seconds. The existence of a translucent ring guaranteed the nerve damage at the crushed spot. After that, 2-4 stitches were used to sew the mice's skin, and povidone-iodine (pyodine) was administered as an antiseptic measure to prevent infection (Hussain *et al*., 2013; Sajid *et al*., 2021).

Study design

Following the sciatic nerve compression, all mice were divided into the following equal groups: The treatment groups were administered *B. utilis* extract at 25 mg/kg (n=8) and 50 mg/kg (n=8), respectively, whereas the control group (group 1, n=8) and sham group (group 2, n=8) were given normal saline. Mice were administered all doses by oral gavage.

Behavior analyses

Muscular force measurement

The muscle grip strength test is used to assess the muscle motor recovery. A horizontal metal grid was used to evaluate the muscular strength of the ipsilateral and contralateral hind limbs. The mice were placed on a horizontal metal grid. A strength meter was used to measure the pulling power for every mouse. To guarantee functional recovery, readings from the normal and experimental groups were compared. Every experiment was carried out three times (Aziz *et al*., 2019).

Walking pattern assessment

The Sciatic Functional Index (SFI) can assess motor function recovery. Ink was applied to the mice's hind limb paws. A white paper was used as the base for a wooden walking track measuring 50 cm. For the experimental (E) and normal (N) sides, the mice with the cleanest ink prints for each run were chosen. SFI was computed using the formula below:

$$SFI = \left(-38.3 \times -\frac{EPL - NPL}{NPL}\right) + \left(109.5 \times \frac{ETS - NTS}{NTS}\right) + \left(13.3 \times \frac{EIT - NIT}{NIT}\right) - 8.8$$

The distance between the heel and the tip of the third toe is known as print length (PL), the distance between the second and fourth toes is known as intermediary toe spread (IT), and the distance between the first and fifth toes is known as toe spread (TS). The IT, TS, and PL of ipsilateral (experimental) paws were EIT, ETS, and EPL, while the IT, TS, and PL of contralateral (normal) paws were NIT, NTS, and NPL (Razzaq *et al*., 2020).

Hotplate test for evaluating thermal response

The hotplate test was used to assess sensory recovery. The time the mice took to leap or lick their rear paws after the

hotplate was heated to 52±0.5 °C was recorded. Hotplate latency (HPL) was the term used to describe this number. Using a stopwatch as the withdrawal reflex (WRL), the latency period for each mouse was determined. After a 2-minute break between each subsequent reading, three readings were obtained. To prevent thermal injury to the mouse, which did not respond for 30 seconds, it was taken away from the heated surface (Kamran *et al*., 2020).

Pinprick test for mechanoception response

Mice's sensory function was evaluated using the pinprick test. Five sections made up the most lateral portion of the hind paw. The plantar areas were then punctured with the pin. It began at each animal's most lateral toe and proceeded to the heel. Mice exhibit a favorable response when they lick their foot or pull their paws away. "1" denoted a positive result in each area. According to Zafar *et al*. (2021), the pinprick score was the total of the results from every region.

Biochemical analyses

Total oxidant status (TOS) of the biological system

The degree of oxidative stress rises in response to illness or injury. During PNIs, this oxidative stress may harm neurons. Oxidative stress levels are closely linked to the total oxidant status (TOS) in living organisms. This test provides an assessment of the overall oxidative stress level in the body. The procedures were carried out according to the protocols established in earlier studies (Aziz *et al.,* 2019). This assay is based on the oxidation of o-Dianisidine ferrous ion into ferric ion by oxidants present in biological samples. Subsequently, xylenol orange forms a colored complex with ferric ions under acidic conditions. The intensity of the sample color, reflecting the oxidant status of the sample, was measured using a spectrophotometer. Hydrogen peroxide H_2O_2, was used as the calibration standard. Oxidative stress levels in each group were therefore estimated using TOS. It is expressed in µmol H_2O_2equiv/L (Imran *et al*., 2019).

Total antioxidant capacity (TAC) of the biological system

The living body is protected by antioxidants from free radicals, which harm the body. The antioxidant ability is the property of living system to defend the living body against free radicals, which cause damage in the body during pathophysiological processes. An optimal antioxidant capacity can support the body's resistance to numerous diseases. The total antioxidant capacity (TAC) was measured to evaluate the antioxidant capacity in serum samples from all experimental mice after dissection. TAC will measure the biological system's antioxidant capacity and be used to assess free radical concentration in mice. It is expressed in mmol Trolox equiv/L (Zafar *et al*., 2020).

Malondialdehyde (MDA)

The thiobarbituric acid technique was employed in this experiment to quantify MDA. 500 µl of TBA agent was added to the diluted sample after 100 µl of the serum sample was mixed with 900 µl of distilled water for the test. Following an hour of cooling at 100 °C, the mixture was centrifuged for ten minutes at 4000 rpm. After separation of the supernatant, the samples' absorbance was measured at 534 nm using a spectrophotometer. The silent factor (105×1.56) is used to compute the MDA concentration, and the results are reported in micromoles per litre.

Muscle weight

Muscle atrophy begins when nerve damage occurs, as the muscles and nerves are no longer connected. Since muscle atrophy is a major limiting factor for functional escalation during PNIs, the mass of the gastrocnemius and tibialis muscles was quantified to assess the extent of this atrophy. The muscle mass of the contralateral and ipsilateral legs was measured after the muscle harvesting and compared to the control group (Razzaq, Hussain, *et al*., 2020).

Random blood glucose

Before and after the procedure, a random blood glucose (glycemic) measurement was obtained. After an injury, hyperglycemia slows healing. Thus, tail blood was collected from mice onto a glucometer strip to determine glycemic levels in each group (Jabeen *et al*., 2023).

Histology (Morphometric analyses)

Following beheading, the ipsilateral and contralateral muscles were removed and preserved in 10% neutral buffer formalin. Each tissue was then fixed in paraffin, and a microtome was used to cut 5 µm sections. Hematoxylin and eosin (H&E) staining was applied after the sections were placed on slides. Lastly, these slices were inspected at 40x using a microscope (AccuScope 3000, USA) that had a 5-megapixel camera. Later, ImageJ version 1.52 was used to quantify the cross-sectional area of each muscle fibre within an equally selected region of the image. A comparison with the control group was made using the mean of all fibers per image (Maqbool *et al*., 2020).

Statistical analysis

All results were recorded as mean ± standard error of the mean (SEM) and were analyzed using GraphPad Prism 8.4.2. Analysis of variance (ANOVA) was used, along with Turkey's multiple comparisons test, to compare means across groups at different time points. A value of $p < 0.05$ was considered statistically significant for all observations.

RESULTS

Determination of safe dose

The mice were orally administered *B. utilis* bark extract at different doses for 2 weeks (25, 50, 100, and 200 mg/kg). These mice were observed daily to assess changes in behavior, feeding patterns, drinking, and weight. In groups treated with *B. utilis* (100 and 200 mg/kg), mortality was observed (Fig. 1) compared to other groups.

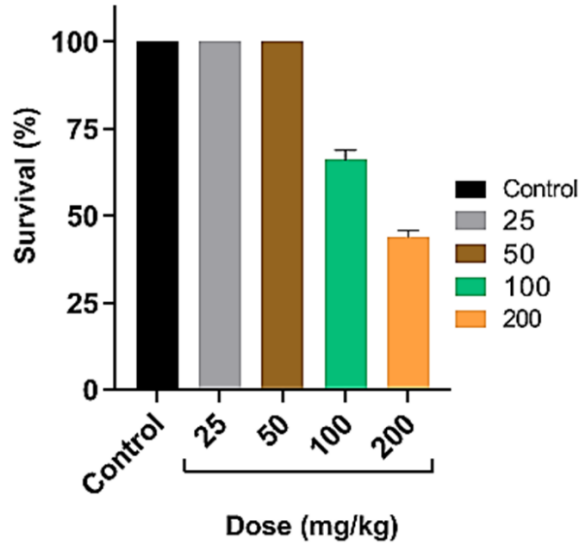

Fig. 1: % Survival of animals in control and treated groups (n=8).

After 2 weeks, the remaining mice were sacrificed and organ histology was performed to assess any changes in the organs. Groups treated with a high dose (100 and 200 mg/kg) showed histopathological changes, but organs of groups treated with a low dose (25 and 50 mg/kg) did not show any significant changes in organ architecture (Fig. 2). Based on overall assessment of groups, mice treated with 25 and 50 mg/kg dose were used for further studies.

Impact of treatment on eating pattern and body weight

To evaluate the effect of *B. utilis* extract on food intake and body weight, we assessed both these parameters throughout the experiment, at different time points. The control (group 1) and the sham group (group 2) were treated with normal saline. Group 3 and group 4 (25 and 50 mg/kg, respectively) were given *B. utilis* extract. The diet intake in all these groups is almost the same in the pre- and post-injury period (Fig. 3a). This shows that *B. utilis* extract did not change the feeding behavior of mice. Moreover, body mass was also assessed throughout the experimental period. *B. utilis* extract showed significant improvement in body weight (Fig. 3b).

Effect of treatment on motor activity

The sciatic nerve plays a dual role in our body, *i.e*., motor as well as sensory functions. It innervates the lower region of the body. Compression of this nerve results in conduction failure to the muscles of the skeletal system, eventually leading to motor function loss in the affected limb (Turkman *et al*., 2023). The gastrocnemius and tibialis muscles are skeletal muscles in the hind limbs, which are innervated by the sciatic nerve. These muscles are involved in running, walking, and flexing the foot inward and outward. After a sciatic nerve crush, the motor endplates of these muscles are denervated, leading to the muscles' inability to contract. Meanwhile, the muscle begins to shrink due to the denervation of that part of the body. Here, motor function recovery was assessed by grip strength, SFI, and gastrocnemius and tibialis muscle mass. The muscle grip strength in the treated group was significantly improved (Fig. 4a). The recovery pattern in SFI was also enhanced in treatment groups (25 and 50 mg/kg) (Fig. 4b). Another parameter to assess the functional recovery is muscle mass. We measured and compared the muscle mass of the ipsilateral and contralateral hind limbs after dissecting them from mice to determine the recovery. Gastrocnemius muscle mass is significantly increased in the 50mg/kg group. In contrast, the 25 mg/kg group did not show a significant effect (Fig. 4c). The tibialis muscle mass improved in both treated groups (25 and 50 mg/kg) (Fig. 4d), but to a slightly lesser extent than in the gastrocnemius.

Effect of treatment on sensory activity

As discussed earlier, the sciatic nerve is a mixed nerve; consequently, mechanical injury results in loss of sensory and motor function. The hotplate and pinprick tests assessed sensory function. These tests are a good means of measuring sensory improvement in mice over the course of treatment and were performed on alternate days throughout the experiment. A significant improvement was observed in all treated groups as compared to the control group. Both treated groups (25 and 50 mg/kg) appeared to be equally effective in showing a statistically significant decrease in paw withdrawal latency from a hot surface on day 7 post injury (Fig. 4a). Similarly, sensory threshold assessment by pinprick test was observed in all treatment groups. The responses of both treated groups (25 and 50 mg/kg) are significant at day 7 and highly significant at day 10 post injury (Fig. 4b).

Effect of treatment on systemic indices

In addition to behavioral assessments, some biochemical analyses were also performed to validate the effectiveness of these plants in promoting axonal regeneration. These analyses also provide clues about the mechanisms underlying improved axonal regeneration. Pathological events during injury may alter blood glucose levels (Fig. 6a). Hyperglycemic conditions can further initiate inflammatory processes and cause tissue necrosis, notably due to increased oxidative stress after injury.

Oxidative stress can be used to assess the pathological phenomena occurring in the body after injury (Fig. 6c & 6d). Following nerve injury, a cascade of reactions generates oxidants that further worsen the injury and delay regenerative processes. Plants contain various phytochemicals, which have antioxidant properties and are effective against oxidants and can initiate the healing of the injured nerve.

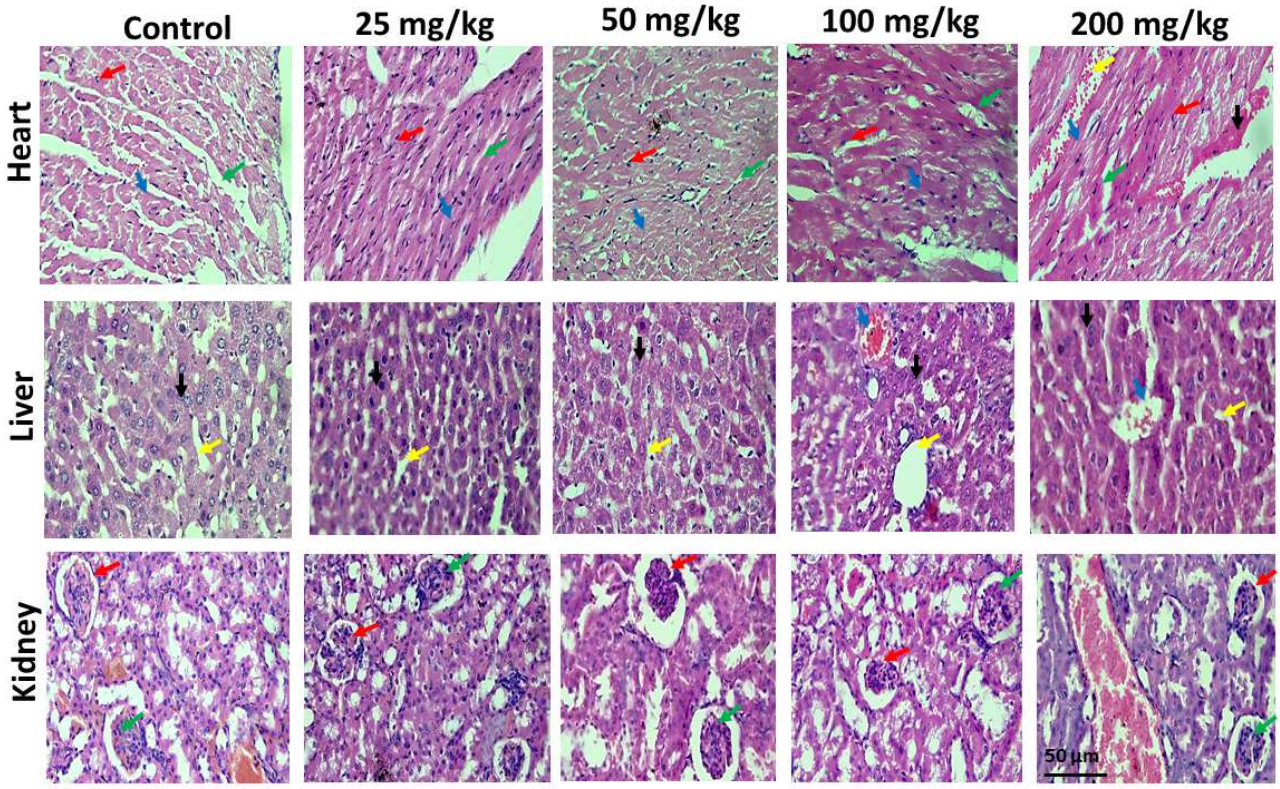

Fig. 2: Histopathological presentation of the vital organs (heart, liver, kidney) taken at 40x magnification, which received different doses of *B. utilis* bark extract.
In the heart, red arrows show the nuclei, blue arrows indicate the intercalated disc, green arrows show the capillary, yellow arrows specify inflammatory cell infiltration, and the black arrow shows cytoplasmic vacuolization. Here, the group treated with high doses revealed varying degrees of cardiotoxicity, including myocardial fiber degeneration, cytoplasmic vacuolization, inflammatory cell infiltration, and focal necrosis, depending on the dose. In the liver, black arrows show the sheets of hepatocytes, yellow arrows indicate sinusoids, and blue arrows show the bile duct. Here, the group treated with high doses showed hepatocellular degeneration, cytoplasmic vacuolization, sinusoidal dilatation, congestion, and mononuclear inflammatory cell infiltration in the portal areas. In some cases, focal necrosis and mild fibrosis were observed. In the kidney, red arrows show Bowman's capsule, and green arrows indicate the glomerulus. Here, the group treated with a high dose showed glomerular atrophy, tubular dilation, epithelial desquamation, cytoplasmic vacuolization, and interstitial inflammation. In more severely affected samples, focal necrosis and congestion of blood vessels were observed. These findings are indicative of nephrotoxic effects associated with the administered compound.

After decapitation, the measurement of TAC and TOS in serum samples of animals was conducted to interpret the possible mechanism behind improved functional recovery (i.e., improved TAC values and reduced TOS values).

Effect of treatment on muscle fiber morphology and size

Traumatic nerve loss, tissue injury, accidents and falls can create an interruption in the nerve pathway and its surrounding environment. In response to the damage, the proximal axon undergoes a process of reinnervation and regeneration. This process works only when the distal target is present; without this target, the nerve cannot find its way to reinnervate the muscle, and over time, muscle atrophy occurs (Adidharma *et al*., 2022). Hence, to assess the histology of muscle reinnervation in each group, a histological assessment was performed.

Histological comparison of the Tibialis anterior muscle from the ipsilateral and contralateral sides in each group was taken (Fig. 7). Each muscle fiber was evaluated entirely, and ImageJ measured a cross-sectional area.

DISCUSSION

PNIs are complicated medical disorders that, if left untreated, can cause lifelong impairments in the patient. Perception, movement, behavior, consciousness, and sensations can all be impacted. In extreme cases, it results in permanent impairments.

The medical community has been concerned about these PNIs for many years. To improve axonal regeneration, neuronal survival, and reinnervation of peripheral targets, various treatment approaches are currently accessible, including non-surgical and surgical procedures (Lopez-Cebral *et al*., 2017). We still lack the best treatments for a complete and flawless functional recovery of the damaged organ, even with the abundance of available possibilities.

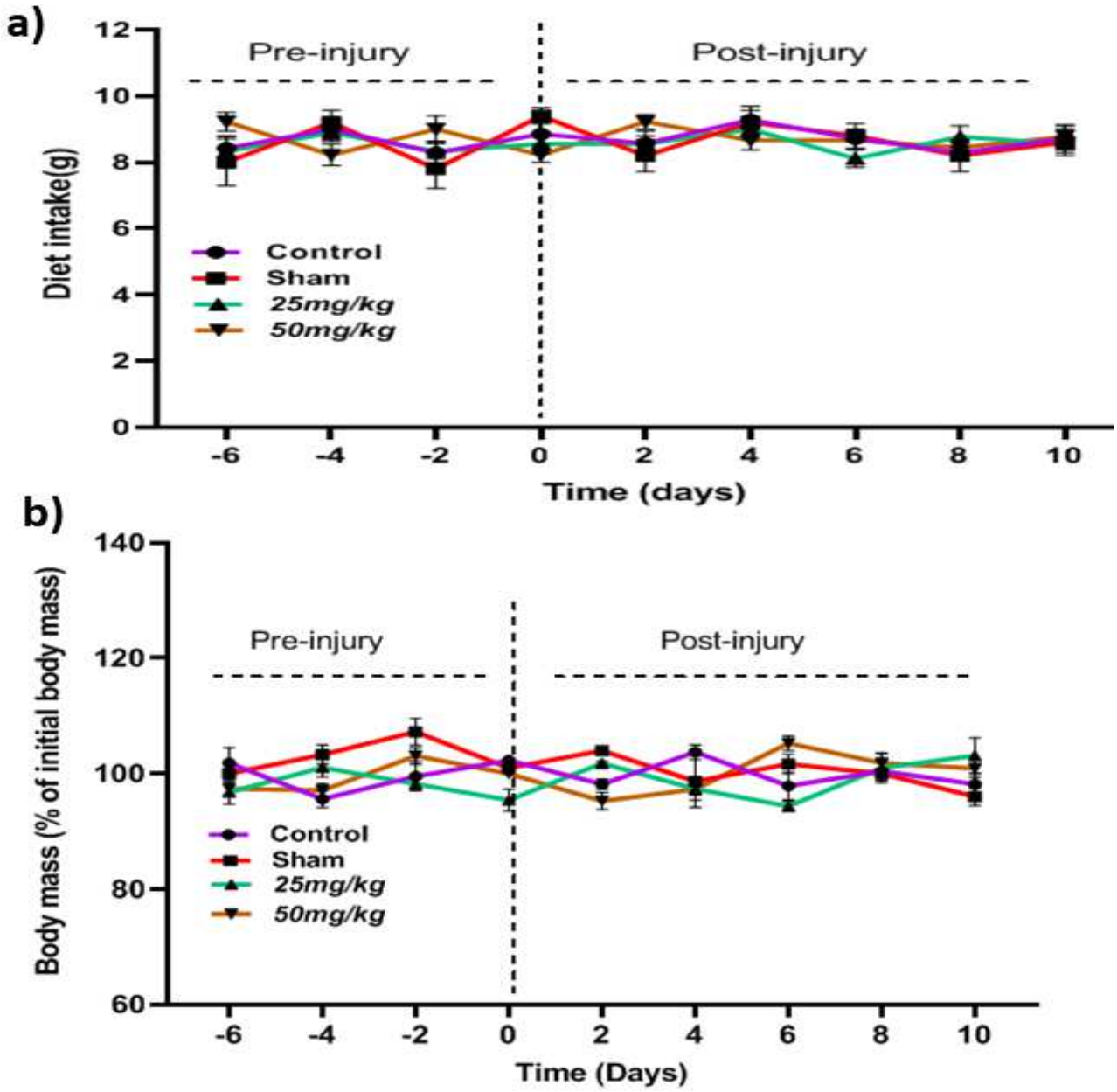

Fig. 3: Effect of *B. utilis* on (a) diet intake in mice treated with normal saline (control and sham: n=8) and *B. utilis* treated groups (25 and 50 mg/kg: n=8). Two-way ANOVA showed a non-significant (p=0.064) effect of *B. utilis* extract on diet intake. (b) Effect of extract on body mass of all mouse groups. Two-way ANOVA showed a significant (p<0.001) effect of treatment on body mass.

Finding such methods or substances that could carry out complete functional recuperation following PNIs is desperately needed in the modern era. In this context, plant-derived chemicals are attracting attention, and several recent studies have confirmed their ability to treat a variety of illnesses, including neuronal injuries. For those with this nerve damage, it may offer hope (Vijayavenkataraman, 2020; Sajid *et al*., 2025).

Furthermore, compared to synthetic molecules, these natural substances typically have the fewest adverse effects (Hussain *et al*., 2020; Hussain *et al*., 2018a; Hussain *et al*., 2018b; Hussain *et al*., 2018; Kizilay*et al*., 2019). Plant extracts can enhance nerve function and conduction, lower inflammation, and stimulate nerve growth. The "amplification effect" during peripheral nerve regeneration is enhanced by Hedysari extract, according to published research. In other words, they promote the development of additional lateral branches from the proximal nerve stump. According to Z. Wang *et al*. (2013), this raises the likelihood of a successful nerve reconnection.

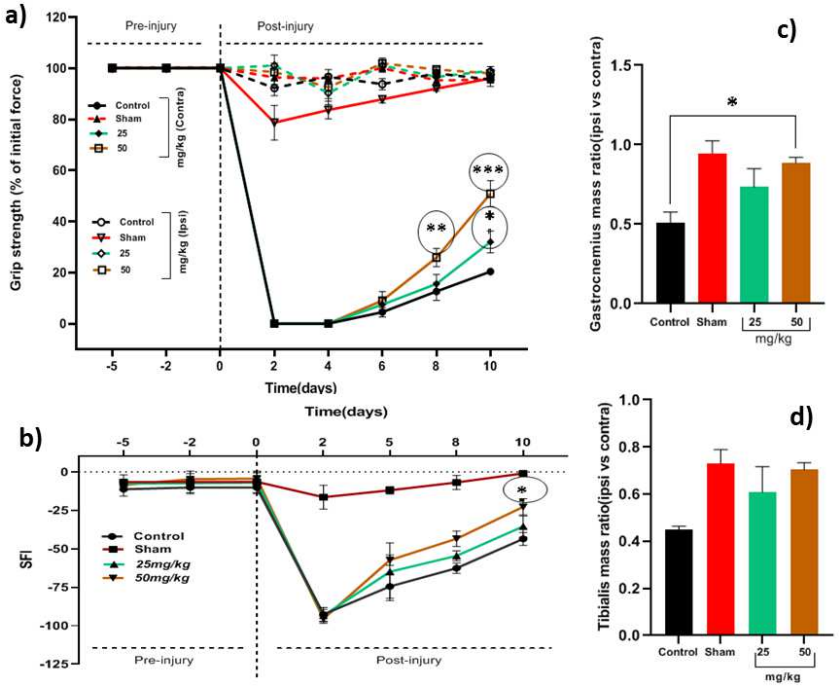

Fig. 4: Effect of *B. utilis* on motor functional recovery after nerve injury.
(a) Time course of muscle grip strength in mice treated with normal saline (control and sham: n=8) and treatment groups (25 and 50 mg/kg: n=8). Measurements were obtained from hind limbs ipsilateral (solid lines) and contralateral (dotted lines) to the injury site. Two-way ANOVA showed a significant (**p<0.001) improvement in the 50mg/kg group, while the 25 mg/kg group showed a non-significant improvement at day 8. At day 10, the 25 mg/kg group showed significant (*p< 0.001) improvement, but the 50 mg/kg group showed highly significant (***p<0.001) results as compared to the control group. (b) Time course of sciatic functional index (SFI) of mice. At day 10, the 50 mg/kg group showed a significant (*p<0.001) improvement, whereas the 25 mg/kg group showed non-significant (p=0.743) results as compared to the control group. (c) Comparison of the gastrocnemius muscle mass of mice; measurements are expressed as a mean muscle mass ratio between hind limbs, ipsilateral and contralateral to the lesion. One-way ANOVA showed a non-significant (p=0.197) difference between the 25 mg/kg group and the control group. At the same time, there is a significant (*p=0.028) difference between the 50 mg/kg group and the control group. (d) Comparison of the anterior tibialis muscle mass of mice: measurements are expressed as a mean muscle mass ratio between hind limbs, ipsilateral and contralateral to the lesion. One-way ANOVA showed a non-significant (p=0.275) difference between the 25 mg/kg group as compared to the control group. There is also a non-significant (p=0.052) difference between the 50mg/kg group and the control group.

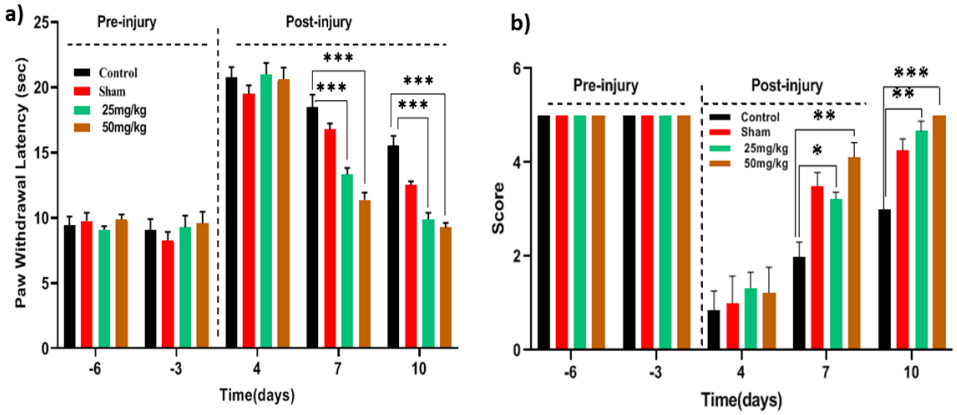

Fig. 5: Effect of *B. utilis* on sensory threshold retrieval after sciatic nerve injury.
(a) Paw withdrawal latency in response to thermal stimulation in mice treated with normal saline (control and sham, n=8) and treated groups (25 and 50 mg/kg, n=8). Measurements were obtained at different time points before and after injury induction. On days 7 and 10, both treated groups (25 and 50 mg/kg) showed a highly significant (***p<0.001) response as compared to the control. (b) Paw withdrawal score in response to pinprick stimulation in mice at day 7: both treatment groups (25 and 50 mg/kg) showed improvement, but the 50 mg/kg group showed (**p< 0.001) better improvement as compared to the 25mg/kg group (*p<0.001). At day 10, 50mg/kg showed highly significant (***p<0.001) results, while the 25 mg/kg group also showed significant (**p <0.001) results as compared to the control group (n=8).

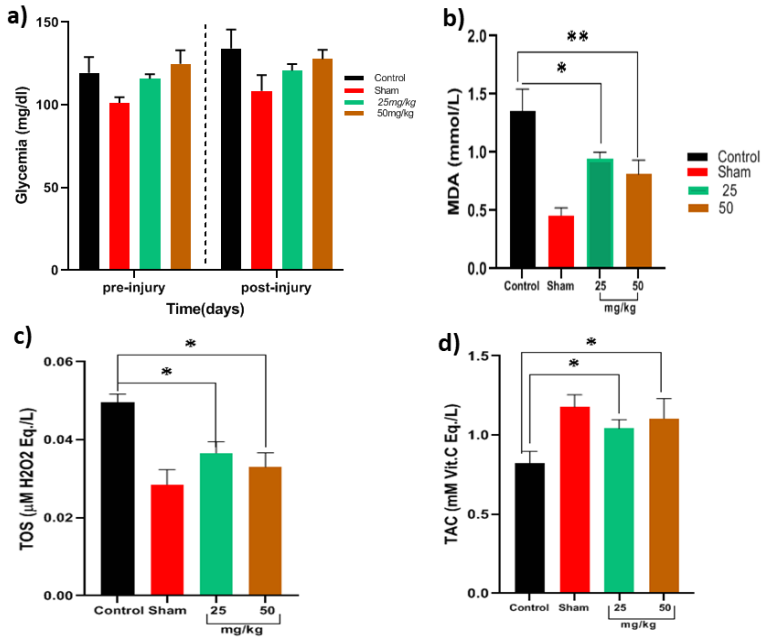

Fig. 6: Effect of *B. utilis* on systemic indices

(a) Glycemic measurement in mice treated with normal saline (control and sham, n=8) and *B. utilis* treated groups (25 and 50 mg/kg, n=8). One-way ANOVA showed a non-significant result (p=0.064) of glucose in the treated and control groups. (b) MDA (Malondialdehyde test), the 25 mg/kg group (n=3) showed a significant (*p=0.016) result, and the 50 mg/kg (n=3) group also showed significant (**p=0.004) results in MDA analysis. (c) In total oxidant status (TOS) of mice, one-way ANOVA showed a significant (*p=0.050) effect in the 25 mg/kg group (n=3), and the 50 mg/kg group (n=3) also showed a significant (*p=0.016) improvement as compared to the control group. (d) Total anti-oxidant capacity (TAC) of mice: One-way ANOVA showed a significant (*p=0.037) effect in the 25 mg/kg group (n=3), whereas the 50 mg/kg group (n=3) also showed a significant (*p=0.011) improvement, and the control group.

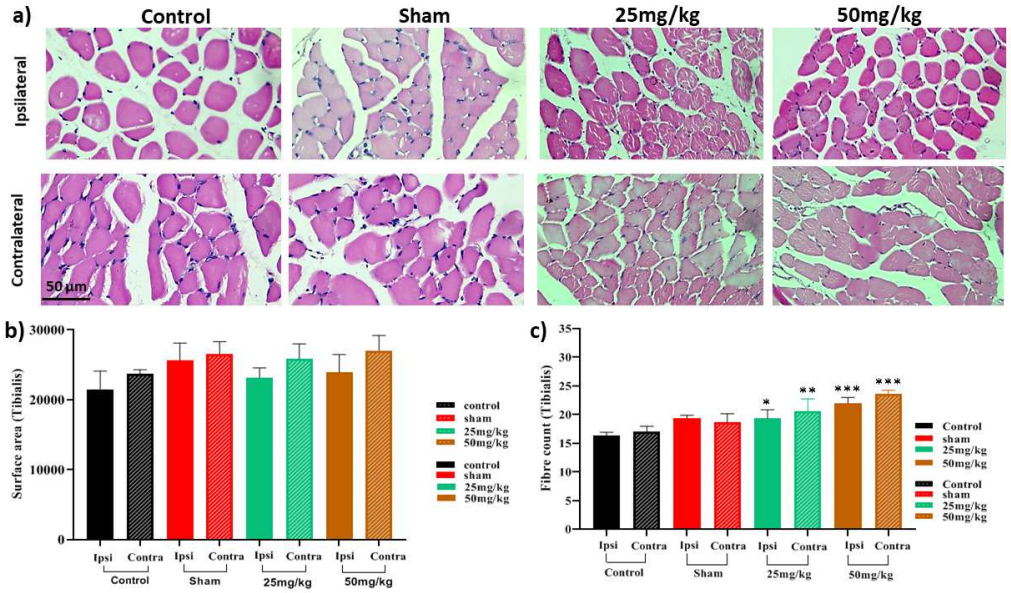

Fig. 7: (a) Histological presentation of the ipsilateral and contralateral muscles of the control, sham, 25, and 50mg/kg groups (n=3). (b) The comparison of the Tibialis surface area between the ipsilateral and contralateral muscles was found to be non-significant (p=0.990) in the 25mg/kg group and 50mg/kg group (one-way ANOVA), also showing non-significant (p=0.933) results as compared to the control group. (c) Whereas the Tibialis fiber count between the ipsilateral and contralateral sides of the treated groups, the 25mg/kg group showed significant (*p=0.043) results, and 50mg/kg was also found to be significant (***p<0.001) as compared to the control group (one-way ANOVA). These findings showed that the treated plant improved muscle dystrophy, retained normal muscle area, and increased fiber count in the treated groups.

One of the most important species in the upper Himalayan regions is *B. utilis*. In addition to flavonoids, tannins, phenolics, triterpenoids and essential oils, the outer layer of the bark includes betulin, betulinic acid, acetyloleanolic acid, sitosterol, lupenone, methyl betulonate and methyl betultriterpenoid (Bhat *et al*., 2022). The bark of birch trees contains a lot of betulin, which has a variety of pharmacological effects, including anticancer, antibacterial, antiviral, antioxidant, anti-inflammatory, anticancerous and carminative qualities. Because betulin has a broad spectrum of biological activities, it has been used in both its unprocessed and converted forms in a variety of industries, including the pharmaceutical and medical ones (Dragicević *et al*., 2022). Traditionally used to cure a variety of medical diseases, such as blood disorders, bacterial infections, ear infections, bronchitis, leprosy, convulsions, etc., *B. utilis* is a prospective research-oriented plant. According to Khanam *et al*. (2023b), different portions of *B. utilis* are delivered via various channels in suitable dosage forms for specific diseases. The current study focuses on evaluating the functional repair of muscles following sciatic nerve injury in a mouse model for the first time, utilizing the varied capabilities of *B. utilis* bark extract.

There is currently no research on mice, although there are a few reports on the dosage assessment of *B. utilis* bark extract. As a result, the ideal dosage was first chosen. The extract was administered orally at four different doses (25, 50, 100 and 200 mg/kg) for testing. Groups with high doses displayed mortality. Betulin, one of the pharmacologically active triterpenoids found in abundance in *B. utilis* bark, can account for as much as 20–30% or even almost 45% of the dry outer bark weight. However, at varying dosages, bark extract and betulin are regarded as safe for rats (Sharma *et al*., 2025). However, the results could be impacted by species variety, the particular plant component used, the extraction technique, the dosage given and the length of exposure. We therefore chose 25 and 50 mg/kg doses to further examine their impact in the nerve injury model based on the observation and to ensure safety.

The sciatic nerve plays a dual role in the body, *i.e*., sensory and motor function. Therefore, different parameters were tested to assess the recovery process. Based on the observation mentioned above, the treated groups accelerate the motor recovery at days 8 and 10. Muscle grip strength showed a more significant (***$p<0.001$) effect in the 50 mg/kg group, and the 25 mg/kg group also showed a significant (*$p<0.001$) effect at day 10 compared to the control group. SFI also revealed significant (*$p<0.001$) effects in the 50 mg/kg group at day 10 compared with other groups. Muscular atrophy is a leading cause of disability after PNIs, and it results in reduced muscle mass and loss of sensory and motor functions. The muscle mass of the gastrocnemius and tibialis improved in the 50 mg/kg-treated group, showing a significant (*$p<0.001$) difference compared with the 25 mg/kg-treated group and the control. This suggests that plant extracts can restore nerve function after nerve injury and help reduce muscle dystrophy. Hotplate and pinprick are sensory responses; both treated groups (25 and 50 mg/kg) showed highly significant (***$p<0.001$) responses in these parameters, indicating that this plant can improve sensory recovery after nerve injury. *Foeniculum vulgare* seed extract loaded into chitosan nanoparticles improved sensory and motor function. Here, phenolic compounds interacted with TRP channels, thereby increasing thermal sensitivity at the site of injury (Bajaber *et al*., 2024).

Furthermore, glucose metabolism is also crucial during PNIs. Numerous harmful metabolic cascades are linked to elevated glucose levels following injury, which may ultimately impede PNI recovery (Roth *et al*., 2023). Furthermore, polyphenols and other substances in natural extracts have been shown to improve insulin sensitivity and secretion, enhance carbohydrate absorption, and regulate blood glucose levels (Bajaber *et al*., 2024). Our results show that following damage, the plant extract preserved the glucose levels.

Because ROS levels vary after PNIs, axon development and regeneration are delayed. According to M. L. Wang *et al*. (2019), oxidative stress is a major contributor to neuronal damage, leading to demyelination, neuroinflammation, apoptosis, and mitochondrial malfunction. Uncontrolled oxidants have been shown to induce fatty acid peroxidation, compromising the structural integrity of biomembranes (Bajaber *et al*., 2024). According to our research, this plant extract significantly improved TAC and TOS. As a result, this extract enhances the healing process and reduces the tension associated with injuries. A naturally occurring triterpenoid, betulinic acid, lowers the amount of malondialdehyde (MDA), increases superoxide dismutase activity, and decreases the buildup of reactive oxygen species. Because it lessens oxidative and nitrosative stress, it also possesses anti-neurodegenerative benefits (Farzan *et al*., 2024). Furthermore, in vascular dementia, betulinic acid has been shown to improve hippocampus neurochemistry and cognitive performance (Huang *et al*., 2022). Muscle mass and function are lost during PNIs. To evaluate muscle degeneration, histopathology of the ipsilateral and contralateral tibialis muscles in the hind limbs was performed. Our results demonstrate that whereas tibialis fiber count was significant ($p<0.001$) in the treated groups (25 and 50 mg/kg), tibialis surface area was not significant ($p=0.560$). These findings demonstrate that this herb helps reinnervate damaged muscles, enhance their function, and treat nerve damage by lowering stress levels. According to our research, a dose of 50 mg/kg is more effective than 25 mg/kg. To fully explore the potential of bark extract, future research is needed on the shape of nerve and muscle fibers, as well as a thorough examination of the molecular processes.

CONCLUSION

According to the current study's findings, *B. utilis* extract speeds up the functional recovery following sciatic nerve injury. The therapeutic potential of *B. utilis* bark extract for treating nerve injury has been validated by recent observations. These results demonstrated that the 50 mg/kg group outperformed the 25 mg/kg group. However, it is essential to mention that doses such as 100 and 200 mg/kg were found to be toxic. Furthermore, investigating the subcomponents of the extracts involved in recovery, as well as the potential mechanisms underlying this enhanced functional recovery, is crucial.

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Author's contribution
Conceptualization: Ghulam Hussain and Muhammad Irfan, Methodology: Usra and Arslan Iftikhar, Investigation: Usra, Arslan Iftikhar and Ghulam Hussain, Writing, reviewing and editing: Ghulam Hussain and Muhammad Irfan, Supervision: Ghulam Hussain

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Data availability statement
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
Following an official examination of the current study design and the use of the rodent model (mouse), the ERC (Ethics Review Committee) of this university (Ref. No. GCUF/ERC/ 641) approved the study.

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

REFERENCES

Adidharma W, Khouri AN, Lee JC, Vanderboll K, Kung TA, Cederna PS and Kemp SWP (2022). Sensory nerve regeneration and reinnervation in muscle following peripheral nerve injury. *Muscle and Nerve,* **66**(4): 384-396.

Aziz N, Rasul A, Malik SA, Anwar H, Imran A, Razzaq A, Shaukat A, Kamran SKS, de Aguilar JLG, Sun T and Hussain G (2019). Supplementation of Cannabis sativa L. Leaf powder accelerates functional recovery and ameliorates haemoglobin level following an induced injury to sciatic nerve in mouse model. *Pak. J. Pharma. Sci.*, **32**(2): 785–792.

Bajaber MA, Hameed A, Hussain G, Noreen R, Ibrahim M, Batool S, Qayyum MA, Farooq T, Parveen B, Khalid T, and Kanwal P (2024). Chitosan nanoparticles loaded with Foeniculum vulgare extract regulate retrieval of sensory and motor functions in mice. *Heliyon*, **10**(3): e25414.

Bhat AM, Qureshi R and Yaseen M (2022). Phytochemical investigation and antioxidant activity of methanolic extract of betula utilis bark. *J. Pharm. Res. Innov.*, **2**(2): 5–17.

Biswasroy P, Pradhan D, Sahu DK, Rai V, Halder J, Rajwar TK, Bhola RK, Kar B, Ghosh G and Rath G (2023). Phytochemical investigation, structural elucidation, *in-silico* study and anti-psoriatic activity of potent bioactive from Betula utilis. *J. Biomol. Struct. Dyn.*, **41**(17): 8093–8108.

Dragicevic A, Kitic D and Pavlovic D (2022). Biological activity of the birch leaf and bark. *Lek. Sirov.*, **42**(1): 89–95.

Farzan M, Farzan M, Shahrani M, Navabi SP, Vardanjani HR, Amini-Khoei H and Shabani S (2024). Neuroprotective properties of betulin, betulinic acid and ursolic acid as triterpenoids derivatives: A comprehensive review of mechanistic studies. *Nutr. Neurosci.,* **27**(3): 223–240.

Huang Y, Zhu Z, Luo C, Ma C, Zhu L, Kong L, Li R, Wu J, Yuan Z and Yi J (2022). Betulinic acid attenuates cognitive dysfunction, oxidative stress and inflammation in a model of T-2 toxin-induced brain damage. *Environ. Sci. & Pollut. Res.*, **29**(34): 52098–52110.

Hussain G, Rasul A, Anwar H, Ahmad Malik S, Maqbool J, Imran A and Suleria HAR (2022). Interventional approaches in peripheral nerve injuries: Pathogenesis and current status. *Bioactive Compounds from Multifarious Natural Foods for Human Health,* Apple Academic Press, pp.255–296.

Hussain G, Wang J, Rasul A, Anwar H, Qasim M, Zafar S, Aziz N, Razzaq A, Hussain R, de Aguilar JLG and Sun T (2020). Current status of therapeutic approaches against peripheral nerve injuries: A detailed story from injury to recovery. *Int. J. Biol. Sci.,* **16**(1): 116–134.

Hussain G, Huang J, Rasul A, Anwar H, Imran A, Maqbool J, Razzaq A, Aziz N, Makhdoom EUH, Konuk M, Sun T (2019). Putative roles of plant-derived tannins in neurodegenerative and neuropsychiatry disorders: An updated review. *Molecules*, **24**(12): 2213.

Hussain G, Rasul A, Anwar H, Aziz N, Razzaq A, Wei W, Ali M, Li J and Li X (2018a). Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *Int. J. Biol. Sci.*, **14**: 341-357.

Hussain G, Schmitt F, Henriques A, Lequeu T, Rene F, Bindler F, Dirrig-Grosch S, Oudart H, Palamiuc L, Metz-Boutigue MH, Dupuis L, Marchioni E, Gonzalez De Aguilar JL and Loeffler JP (2013). Systemic downregulation of Delta-9 desaturase promotes muscle oxidative metabolism and accelerates muscle function

recovery following nerve injury. *PLoS One*, **8**(6): e64525.

Hussain G, Zhang L, Rasul A, Anwar H, Sohail MU, Razzaq A, Aziz N, Shabbir A, Ali M and Sun T (2018b). Role of plant-derived flavonoids and their mechanism in attenuation of Alzheimer's and Parkinson's diseases: An update of recent data. *Molecules*, **23**(4): 1-26.

Imran A, Xiao L, Ahmad W, Anwar H, Rasul A, Imran M, Aziz N, Razzaq A, Arshad MU, Shabbir A, Gonzalez de Aguilar JL, Sun T and Hussain G (2019). *Foeniculum vulgare* (Fennel) promotes functional recovery and ameliorates oxidative stress following a lesion to the sciatic nerve in mouse model. *J. Food Biochem.*, **43**(9): e12983.

Ishfaq B, Khan IU, Khalid SH and Asghar S (2023). Design and evaluation of sodium alginate-based hydrogel dressings containing *Betula utilis* extract for cutaneous wound healing. *Front. Bioeng. Biotechnol.* **26**(11): 1042077.

Jabeen Z, Bukhari SA, Malik SA, Hussain G and Kamal, S. (2023). Improved gut microbiota escalates muscle function rehabilitation and ameliorates oxidative stress following mechanically induced peripheral nerve injury in mice. *Pak. Vet. J.* **43**(4): 707–713.

Juckett L, Saffari TM, Ormseth B, Senger JL and Moore AM (2022). The effect of electrical stimulation on nerve regeneration following peripheral nerve injury. *Biomolecules,* **12**(12): 1856.

Kamran SKS, Rasul A, Anwar H, Irfan S, Ullah KS, Malik SA, Razzaq A, Aziz N, Braidy N and Hussain G (2020). *Ferula asafoetida* Linn. is effective for early functional recovery following mechanically induced insult to the sciatic nerve of a mouse model. *Trop. J. Pharm. Res.*, **19**(9): 1903–1910.

Khanam A, Gupta R, Kumari M and Gupta A (2023a). Diverse pharmacological potentials of betula utilis: A critical review. *Int. J. Pharm. Sci. Med.* **8**(4): 101–109.

Kizilay Z, Aktas S, Kahraman Cetin N, Kilic MA and Ozturk H (2019). Effect of *Tarantula cubensis* extract (Theranekron) on peripheral nerve healing in an experimental sciatic nerve injury model in rats. *Turk. Neurosurgery*, **29**(5): 743–749.

Kumar Vishwakarma R, Negi A and Singh Negi D (2022). Antioxidant, COX inhibition activity of different extract of bark of *Betula utilis* and molecular docking analysis of its phytochemicals against COX-1 and COX-2 isoenzyme. *Mater. Today. Proc.* **57**: 251–

Lopez-Cebral R, Silva-Correia J, Reis RL, Silva TH and Oliveira JM (2017). Peripheral nerve injury: Current challenges, conventional treatment approaches and new trends in biomaterials-based regenerative strategies. *ACS Biomater. Sci. Eng.,* **3**(12): 3098–3122.

Maqbool J, Anwar H, Iqbal J, Rasul A, Imran A, Ahmad Malik S, Shabbir A, Ijaz F, Sajid F, Akram R, Sun T, Imran M, Hussain G and Islam S (2020). Methanolic extract of Fennel (*Foeniculum vulgare*) escalates functional restoration following a compression injury to the sciatic nerve in a mouse model. *Food. Sci. Nutr.*, **13**(2): 701-710.

Marrelli M (2021). Medicinal plants. *Plants,* **10**(7): 1355.

Modrak M, Talukder MAH, Gurgenashvili K, Noble M and Elfar JC (2020). Peripheral nerve injury and myelination: Potential therapeutic strategies. *J. Neurosci. Res.,* **98**(5): 780–795.

Pan D, Mackinnon SE and Wood MD (2020). Advances in the repair of segmental nerve injuries and trends in reconstruction. *Muscle and Nerve,* **61**(6): 726–739.

Razzaq A, Ahmad Malik S, Saeed F, Imran A, Rasul A, Qasim M, Zafar S, Kamran SKS, Maqbool J, Imran M, Hussain G and Hussain M (2020). *Moringa oleifera* Lam. ameliorates the muscles function recovery following an induced insult to the Sciatic nerve in a mouse model. *Food. Sci. Nutr.* **8**(8): 4009–4016.

Razzaq A, Hussain G, Rasul A, Xu J, Zhang Q, Malik SA, Anwar H, Aziz N, Braidy N, de Aguilar JLG, Wei W, Li J and Li X (2020). *Strychnos nux-vomica* L. seed preparation promotes functional recovery and attenuates oxidative stress in a mouse model of sciatic nerve crush injury. *BMC Complement. Med. Ther.,* **20**(1): 181.

Roth E, Linehan A, Weihrauch D, Stucky C, Hogan Q and Hoben G (2023). Targeted muscle reinnervation prevents and reverses rat pain behaviors after nerve transection. *Pain*, **164**(2): 316–324.

Sajid F, Rasul A, Imran A and Malik SA (2021). Indian ginseng (n. hexane and chloroform extracts) offers ameliorating effects on muscle functions restoration in a mouse model of peripheral nerve injury. *Int. J. Biosci.* **6655**: 231–240.

Sajid F, Zubair M, Iftikhar A and Hussain G (2025). Dietary supplementation with *Withania somnifera* root powder promotes recovery after peripheral nerve injury in mice. *Pak. J. Pharm. Sci*., **38**(6): 2111-2119

Sharma S, Sharma D and Dhobi M (2025). An updated overview on traditional uses, phytochemistry and pharmacological properties of *Maytenus emarginata* (Willd.) Ding Hou (1981–2024). *Discover Plants*, **2**(1): 1–20.

Shoaib S, Ansari MA, Fatease A Al, Safhi AY, Hani U, Jahan R, Alomary MN, Ansari MN, Ahmed N, Wahab S, Ahmad W, Yusuf N and Islam N (2023). Plant-derived bioactive compounds in the management of neurodegenerative disorders: Challenges, future directions and molecular mechanisms involved in neuroprotection. *Pharmaceutics,* **15**(3): 749.

Sonawane K, Dixit H, Thota N, Mistry T and Balavenkatasubramanian J (2023). Knowing it before blocking it, the ABCD of the peripheral nerves: Part B (Nerve injury types, mechanisms and pathogenesis). *Cureus*, **15**(8): e43143.

Turkman A, Thanaraaj V, Soleimani-Nouri P, Harb E and Thakkar M (2023). Outcomes of sciatic nerve injury repairs: A systematic review. *Eplasty*, **23**: e42.

Vijayavenkataraman S (2020). Nerve guide conduits for peripheral nerve injury repair: A review on design,

materials and fabrication methods. *Acta Biomater.,* **106**: 54–69.

Wang ML, Rivlin M, Graham JG and Beredjiklian PK (2019). Peripheral nerve injury, scarring and recovery. *Connect. Tissue. Res.* **60**(1): 3–9.

Wang Z, Zhang P, Kou Y, Yin X, Han N and Jiang B (2013). Hedysari extract improves regeneration after peripheral nerve injury by enhancing the amplification effect. *PLoS ONE*, **8**(7).

Zafar S, Anwar H, Qasim M, Irfan S, Maqbool J, Sajid F, Naqvi SAR and Hussain G (2020). *Calotropis procera* (root) escalates functions rehabilitation and attenuates oxidative stress in a mouse model of peripheral nerve injury. *Pak. J. Pharma. Sci.* **33**(6): 2801–2807.

Zafar S, Rasul A, Iqbal J, Anwar H, Imran A, Jabeen F, Shabbir A, Akram R, Maqbool J, Sajid F, Arshad MU, Hussain G and Islam S (2021). *Calotropis procera* (leaves) supplementation exerts curative effects on promoting functional recovery in a mouse model of peripheral nerve injury. *Food. Sci. Nutr.,* **9**(9): 5016–5027.