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Alleviating doxorubicin-induced neurotoxicity with roselle extract

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ABSTRACT

Doxorubicin (DOX) is a potent chemotherapeutic drug used in cancer treatment, but it can lead to neurotoxicity particularly in the peripheral nervous system causing neuropathic pain. Roselle extract, known for its neuroprotective properties, has gained attention as a potential therapeutic agent in mitigating such neurotoxic effects. In this study investigated the neuroprotective potential of roselle water extract (RWE) against DOX-induced neurotoxicity in rats, focusing on the spinal cord, sciatic nerve, and neuropathic pain. Male albino rats were divided into four groups (6 rats/group); control, RWE-treated (500 mg/kg body weight/day; oral adminstration), DOX-treated (cumulative dose of 18 mg/kg body weight; intraperitoneal injected), and DOX + RWE-treated groups. Various assessments, including thermal nociception tests (hot plate, tail flicking, and cold plate), biochemical analysis of oxidative stress markers including malondialdehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and histopathological evaluation of spinal cord and sciatic nerve tissues were conducted. DOX treatment led to increases in oxidative stress (↑MDA, ↑MPO, ↓SOD, and ↓CAT), neuronal damage, axonal demyelination, astrocytes number, and microglial cell activation, and a reduction in Schwann cell population. However, concurrent administration of RWE with DOX significantly reduced oxidative stress, preserved neuronal morphology, decreased glial cells activation, improved Schwann cell density, and attenuated neuropathic pain responses compared to the DOX-only treated rats. These findings suggested that RWE possessed antioxidant and neuroprotective properties, making it a potential complementary therapy to alleviate peripheral neuropathy associated with DOX treatment.

Key words: Doxorubicin, Neurotoxicity, Roselle Extract, Spinal cord, Sciatic nerve

1. Introduction

Neurotoxicity occurs when dangerous substances (neurotoxicants) impair the central, peripheral, and autonomic nervous systems (Serap, 2022). Doxorubicin (DOX), a potent anthracycline chemotherapeutic drug, has revolutionized cancer treatment by effectively targeting rapidly dividing cancer cells (Kamińska and Cudnoch-Jędrzejewska, 2023). However, its use comes with a price – the potential to induce neurotoxicity (Khadrawy et al., 2021). Over 50% of children with cancer who receive anthracycline-based regimens are at risk of developing lifelong neurological damage (Kamińska and Cudnoch-Jędrzejewska, 2023).

The peripheral nervous system is more vulnerable to external agents and medication toxicity, particularly from chemotherapy, due to its lower level of protection compared to the central nervous system (Yardim et al., 2022). DOX has low brain penetration due to the blood-brain barrier. Thus, peripheral activation causes

most of its toxicity (Kamińska and Cudnoch-Jędrzejewska, 2023). DOX disrupts mitochondrial membranes, reducing energy production, and increasing reactive oxygen species (ROS) production, resulting in redox imbalance leading to neuron and glial cells damage (Kamińska and Cudnoch-Jędrzejewska, 2023). This disruption can ultimately result in neurotoxicity and contribute to the development of encephalopathies, cognitive impairment, and peripheral neuropathy (Imosemi et al., 2022; Khadrawy et al., 2021).

Chemotherapy-induced peripheral neuropathy is a common neurotoxic side effect observed with various anticancer agents, including DOX (Farshid et al., 2015; Yardım et al., 2021a). It affects approximately 68% of cancer patients within the first six months of treatment. Symptoms include paresthesia, allodynia, and neuropathic pain, significantly impacting patients' quality of life (Semis et al., 2021; Selvy et al., 2022) . The origin of peripheral neuropathy involves the spinal cord dorsal horn, dorsal root ganglion, and peripheral nerves like the sciatic nerve injury (Brandolini et al., 2019). Previous investigations reported the neurotoxic effects of DOX on the spinal cord dorsal root ganglion (Mahmoodazdeh et al., 2020) and sciatic nerve (Farshid et al., 2015). However, very limited data are available to provide valuable insights into DOX-mediated mechanisms underlying peripheral neuropathy. Developing strategies to protect the nervous system could significantly enhance the well-being of cancer patients undergoing DOX treatment. Peripheral neuropathy and neurotoxicity are significant side effects for oncologists treating cancer patients, leading to growing interest in potential neuroprotective agents (Yardım et al., 2021b). While traditional pharmacological treatments (like anti-inflammatory drugs, antihistamines, antidepressants, anti-convulsants, non-steroidal, and opioids) are used to manage neuropathic pain, but their effectiveness is limited (Yaşar et al., 2020). This highlights the urgent need for new therapeutic approaches.

Recently, natural compounds have gained attention for their potential neuroprotective properties. One such compound is *Hibiscus sabdariffa*, also known as roselle, karkade, sour tea, or red sorrel that is widely cultivated in tropical areas (Oboh et al., 2018; Izquierdo-Vega et al., 2020). This herb, belonging to the Malvaceae family, is rich in acids (malic acid, citric acid, and tartaric acid), polyphenols, alkaloids, tannins, flavonoids, carotenoids, steroids, anthocyanins, organic acids, polysaccharides, vitamin B complex, and vitamin C (Shalgum et al., 2019; Izquierdo-Vega et al., 2020; Elegbeleye et al., 2022; Efosa et al., 2023). These compounds are associated with various therapeutic benefits, including antioxidant, anti-inflammatory, anticancer, hepatoprotective, and anti-neurodegenerative effects (; Izquierdo-Vega et al., 2020; Mezni et al., 2020; Montalvo-González et al., 2022; Efosa et al., 2023). Its calyx is commonly used to make beverages and infusions, offering potential health advantages and promoting overall well-being (Oboh et al., 2018). These properties make roselle a valuable natural remedy for various neurological conditions.

The current study aimed to investigate the neuroprotective potential of roselle extract against DOX-induced neurotoxicity in rats, focusing on the spinal cord, sciatic nerve, and neuropathic pain. It also aimed to uncover mechanisms for potential as a natural remedy for preventing chemotherapy-induced peripheral neuropathy.

2. Materials and methods

Chemicals

The DOX vials, marketed as Adricin®, were obtained from Hikma Pharmaceuticals in Egypt. Each vial contains a 100 mg/25 ml injectable solution. Assay kits for malondialdehyde (MDA) and myeloperoxidase (MPO) were sourced from Elabscience® (catalog numbers: E-BC-K025-S and E-BC-K074-M, respectively), while the superoxide dismutase (SOD) and catalase (CAT) assay kits were obtained from Biodiagnostic (catalog numbers: SD 25 21 and CA 25 17, respectively). All substances and reagents were of analytical grade.

Roselle water extract preparation

Roselle (*Hibiscus sabdariffa L.*) was bought from local market and authenticated by Dr. Shaimaa Gamal Salama, Botany and Microbiology Department, Faculty of Science, Damanhour

University. The dried red calyx was procured from a local market and processed into a fine powder. The powder was infused in hot distilled water, filtered, and concentrated (Kasimu et al., 2021). The concentrated roselle water extract (RWE) was then dried and stored at –4°C until use.

Animals

Twenty-four male albino rats (*Rattus norvegicus*) (aged 8-10 weeks, weighing 133-141 g) were acquired from the Giza, National Research Center, Egypt. They were housed in controlled conditions (23 ± 2°C; 45 ± 5% humidity, 12:12 h light/dark cycle) with unrestricted access to food and water, following ethical approval from the Animal Experiments Local Ethics Committee of Damanhur University (Approval No. DMU-SCI-CSRE-23-12-02).

Group allocation

The rats were allocated randomly into four groups, with six rats in each group (n=6 in each group). Control group (G1): Non-treated rats. Roselle water extract group (RWE, G2): Rats received orally 500 mg/kg body weight/day of roselle water extract (Kasimu et al., 2021). DOX group (DOX, G3): Rats received a cumulative dose of DOX (18 mg/kg body weight, intraperitoneal) administered in six equal doses (3 mg/kg) every other day for three weeks (Alafifi et al., 2023). DOX + roselle water extract group (DOX + RWE, G4): Rats received an intraperitoneal DOX (3 mg/kg body weight) every other day alongside daily oral roselle water extract.

Assessment of thermal nociception in rats

At the end of the three-week experimental duration, rats underwent thermal nociception assessment. Thermal hyperalgesia (increased sensitivity to painful stimuli) was evaluated using the hot plate and tail immersion tests, while the cold plate test examined cold sensitivity and allodynia (pain from non-painful stimuli) (Deuis et al., 2017).

Hot plate test

Rats were placed on a 52°C plate, and the time until they showed heat sensitivity (hind paw licking, lifting, or jumping) was measured as latency time, with a 40-second cutoff to prevent tissue damage (Semis et al., 2022).

Tail flick test

One-third of rat tails were in a 49-50°C water path, and the time until they showed heat sensitivity (tail flicking, withdrawal, or struggle) was measured as latency time, with a 12-second cutoff to prevent tissue damage (Liu et al., 2021).

Cold plate test

Rats were placed on a 4°C plate, and the time until they showed cold sensitivity (paw lifting, licking, or shaking) was measured as latency time, with a 40-second cutoff to prevent tissue damage (Semis et al., 2022).

Tissue collection

After completion of thermal nociception tests, rats were euthanized using intraperitoneal injection of 60 mg/kg sodium pentobarbital. After anesthesia, blood was collected from the heart through a small incision in the thoracic cavity, using a syringe to draw blood into a serum tube. Then, the thoracolumbar spinal cord and sciatic nerve tissues were dissected using surgical instruments, gently handled to avoid damage, and placed in a 10% formalin solution.

Biochemical analysis of serum samples

The level of malondialdehyde (MDA) was quantified based on the generation of a red color resulting from the interaction between thiobarbituric acid and MDA (Ohkawa et al., 1979). Results were measured at 532 nm using a spectrophotometer and listed as nmol/ml. The myeloperoxidase (MPO) activities were quantified based on the oxidation of O-dianisidine dihydrochloride by hydrogen peroxide (Krawisz et al., 1984). Results were measured at 460 nm using a spectrophotometer and listed as µU/ml. The superoxide dismutase (SOD) activities were quantified based on the inhibition of nitroblue tetrazolium dye by phenazine methosulphate (Nishikimi et al., 1972). Results were measured at 560 nm using a spectrophotometer and listed as U/ml. The catalase "CAT" activities were quantified based on Aebi (1984) using H_2O_2 as a substrate. Results were measured at 570 nm using a spectrophotometer and listed U/ml.

Light microscopic sample preparation

The spinal cord and sciatic nerve samples from different experimental groups were fixed in 10%

formalin for 24 hours, washed in water for 18 hours, dehydrated in ethanol, cleared in xylene, and embedded in paraffin (Bancroft and Layton, 2019). Sections were cut at 5 μm thickness, stained with hematoxylin-eosin, and photographed and evaluated for histopathological changes using Olympus CX41 microscope.

Statistical Analysis

The data was verified using GraphPad Prism version 5.01, with statistical significance assessed using ANOVA and Tukey's *post-hoc* tests and presented as mean ± standard deviation (SD).

Results

Thermal nociception assessment

Heat hyperalgesia

The thermal nociception assessment involved both the hot plate and tail flicking tests for heat hyperalgesia. Paw withdrawal and tail withdrawal latency differed significantly among groups ($p < 0.01$) (Figure 1A&B). DOX-treated rats (G3) had shorter paw and tail withdrawal latency times compared to the controls (G1) and RWE-treated rats (G2), indicating thermal hyperalgesia and confirming neuropathic pain. Concurrent administration of DOX+RWE (G4) caused a partial increase in paw and tail withdrawal latency times compared to the DOX-treated rats (G3), indicating partial attenuation of thermal hyperalgesia. However, G4 also had slightly shorter latencies than controls (G1) and RWE-treated rats (G2).

Cold sensitivity and allodynia

Rats exposed to the cold plate showed signs of discomfort such as hand withdrawal, shivering, and scratching at different latency times (Figure 1C). DOX-treated rats (G3) had significantly shorter latencies to hand withdrawal and shivering ($p<0.05$) than controls (G1) and RWE-treated rats (G2), confirming cold allodynia and neuropathic pain. Concurrent administration of DOX+RWE (G4) caused a significant increase in latencies ($p<0.05$) compared to the DOX-treated rats (G3), indicating attenuation of cold allodynia. However, G4 still had slightly shorter latencies than controls (G1) and RWE-treated rats (G2) ($p>0.05$).

Biochemical parameters analysis

Oxidative stress biomarkers

Analysis of serum MDA level and MPO activities revealed significant differences among the experimental groups (Fig. 2 A and B). Significant elevations in the level of MDA and MPO activity were detected in the DOX-treated group (G3) as compared with the control (G1) and RWE-treated group (G2). Concurrent administration of DOX+RWE (G4) resulted in a highly significant decrease in MDA level and MPO activity ($p<0.001$) compared to the DOX-treated group (G3). The group (G4) also showed a significant elevation in MDA level and MPO activity ($p<0.05$) compared to the control (G1) and RWE-treated group (G2).

Antioxidant enzyme activity

The statistical analysis of serum SOD and CAT activities indicated significant variations among the experimental groups (Fig. 2 C and D). Significant reductions in the SOD and CAT activities ($p<0.01$) were observed in the DOX-treated group (G3) as compared with the control (G1) and RWE-treated group (G2). Concurrent administration of DOX+RWE (G4) resulted in significant elevations in SOD and CAT activities ($p<0.05$) compared to the DOX-treated group (G3). The group (G4) also showed significant elevations in SOD and CAT activities ($p<0.05$) compared to the control (G1) and RWE-treated group (G2).

Histopathological evaluation

Spinal cord histological observation

Control (G1) and RWE-treated rats (G2) displayed normal spinal cord morphology with intact gray and white matter structures. The gray matter contained various neurons and neuroglial cells surrounded by neuropil (Fig. 3A and B). Neurons were of different sizes, with large multipolar neurons and smaller interneurons connecting them**.** Neuroglial cells included satellite cells surrounding neurons, oligodendrocytes with rounded dark nuclei, large star shape astrocytes, and microglial cells (Fig. 4A and C). White matter comprised myelinated nerve fibers with myelin sheaths and a few neuroglial cells. Oligodendrocytes were the main glial cells in white matter, along with a few astrocytes and microglial cells near blood vessels (Fig. 4.B and D).

In contrast, DOX-treated rats (G3) showed mild damage in both gray and white matter, with a significant increase in astrocytes and microglial cells numbers compared to the control and RWE-treated rats (Fig. 3C and E). Some neurons were lost, replaced by cavities that enclosed remnants of degenerated neurons and there was neuropil vacuolization (Fig. 4E). White matter exhibited demyelinated fibers and increased astrocytes and microglial cells presence (Fig. 4F). DOX+RWE-treated rats (G4) exhibited preserved gray and white matter architecture, with a significantly lower number of astrocytes and microglial cells compared to the DOX-treated group (G3) (Fig. 3 D and E). In grey matter, normal neurons were observed with very few degenerated neurons (Fig. 4G). White matter mostly retained normal myelinated fibers, with minor damaged areas (Fig. 4H).

Sciatic nerve histological observation

Control (G1) and RWE-treated rats (G2) had normal sciatic nerve morphology with well-organized nerve fascicles surrounded by connective tissue layers. These layers include the endoneurium, which surrounds individual nerve fibers, the perineurium, which surrounds bundles of nerve fibers, and the epineurium, which encases the entire nerve (Fig. 5A-D). Most nerve fibers were myelinated, characterized by axoplasm surrounded by a clear myelin sheath, along with a few unmyelinated fibers showing smaller diameters and an absence of a myelin sheath. Numerous Schwann cells were observed interspersed between the nerve fibers (Fig. 5B and D).

DOX-treated rats (G3) exhibited disorganized nerve fascicles with wide separations from each other and overlying perineurium, and an increased number of small pale demyelinated fibers (Fig. 5 E and F). Nerve fibers suffer from degeneration marked by axonal swelling, axoplasm vacuolization (Fig. 5E), along with significant reduction in Schwann cells number compared to the control (G1) and RWE-treated rats (G2) (Fig. 6).

DOX+RWE-treated rats (G4) showed preserved sciatic nerve architecture; most nerve fibers appeared myelinated fibers, with few degenerated nerve fibers in between (Fig. 5H). The number of Schwann cells was significantly higher compared to the DOX-treated group (G3) marking normal nerve fiber myelination (Fig. 6).

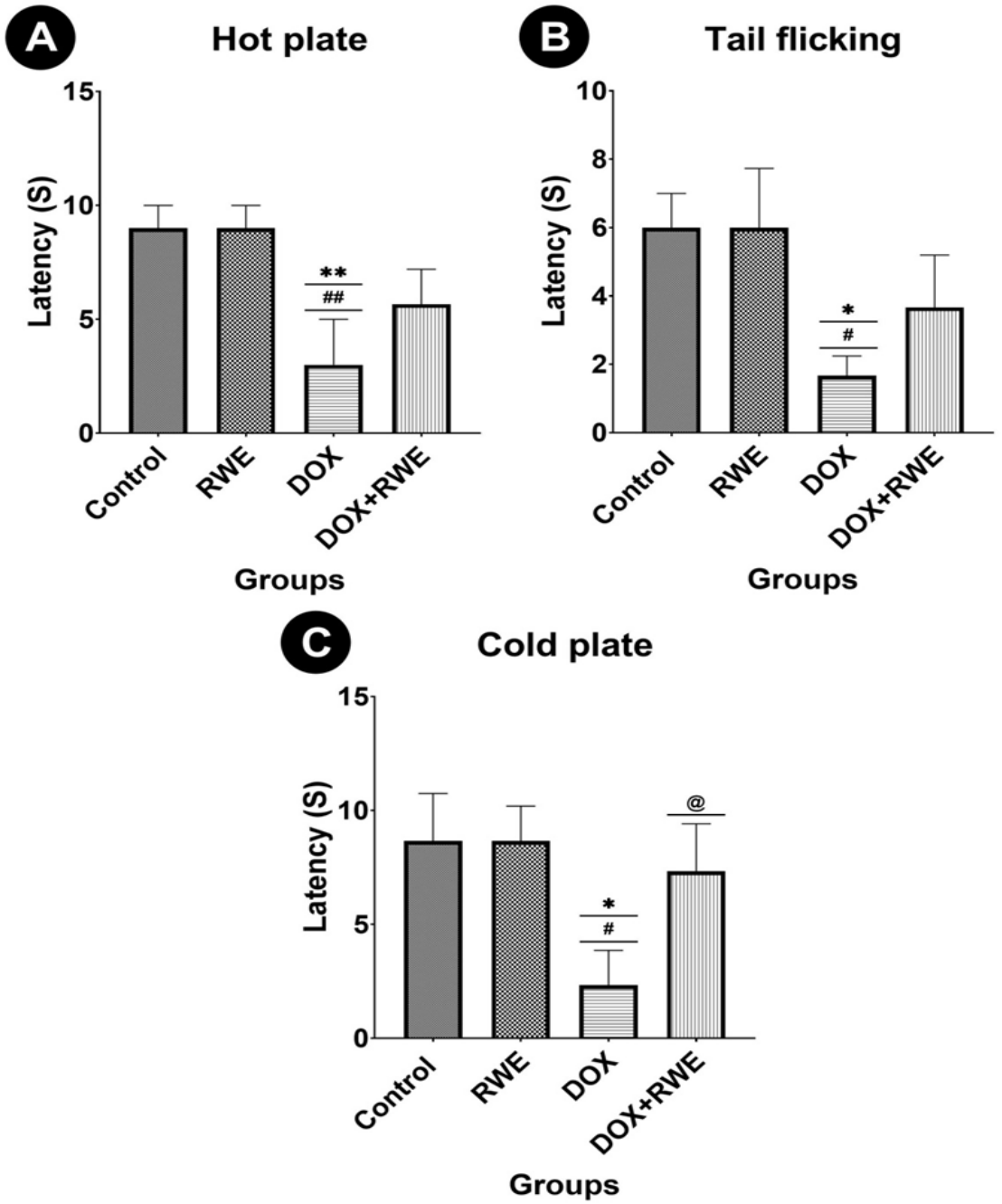

Fig.1. Effect of DOX and RWE on thermal nociception in rats. DOX treatment caused enhancement of pain sensitivity as measured by a decrease in latencies required to elicit a paw withdrawal or tail movement compared to the control and RWE-treated group, indicating thermal hyperalgesia and cold allodynia. Concurrent DOX+RWE showed partial attenuation of thermal hyperalgesia and cold allodynia marked by increased latencies compared to the DOX-treated rats. Data are expressed as mean ± standard deviation. *, ** Indicate significance *vs*. control (*$p < 0.05$, **$p < 0.01$), #, ## indicate significance *vs*. RWE-treated groups (#$p < 0.05$, ##$p < 0.01$), and @ indicates significance *vs*. DOX-treated groups (@$p < 0.05$).

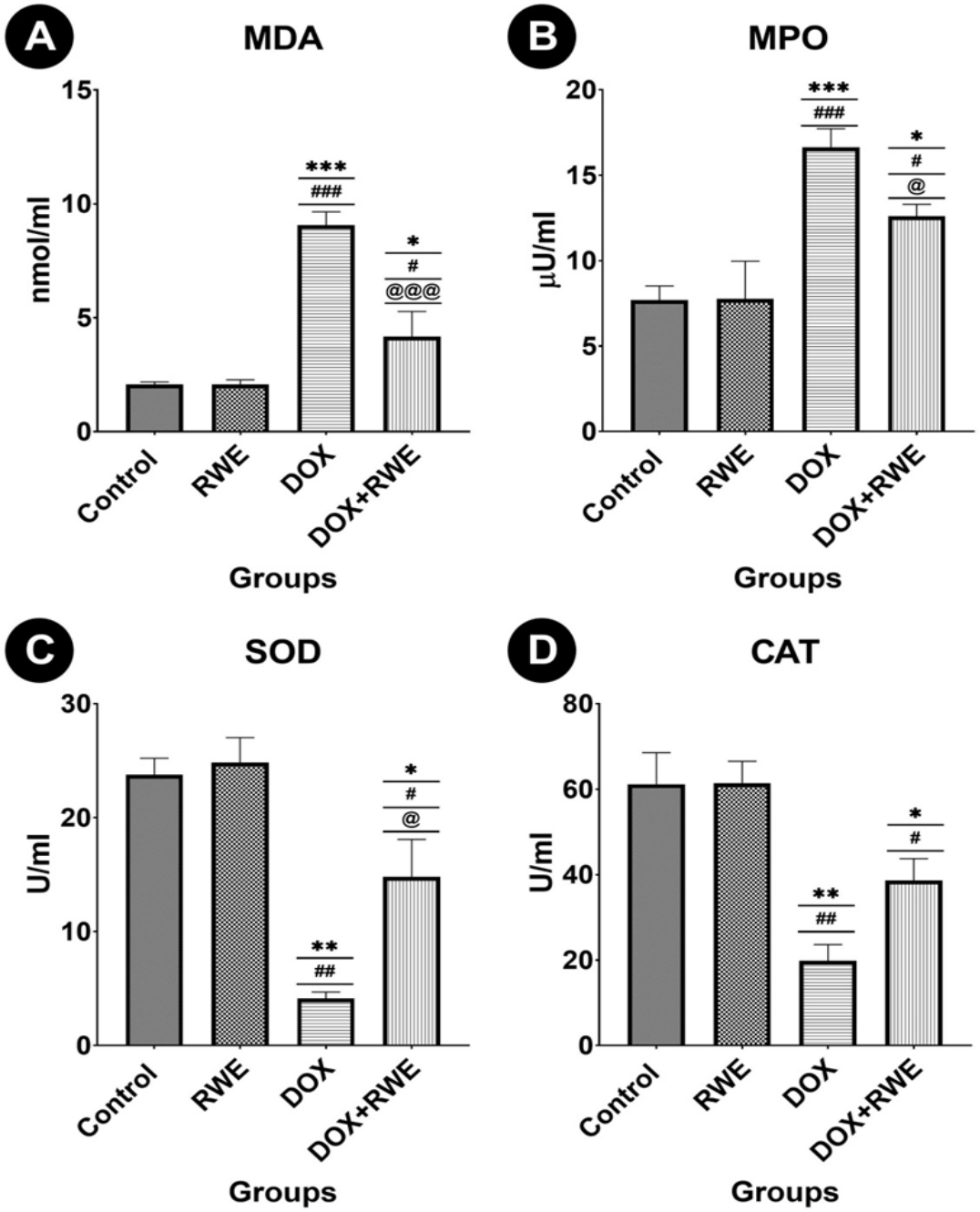

Fig. 2. Effect of DOX and RWE on MDA serum level, and MPO, SOD and CAT serum activities. DOX treatment significantly increased MDA level and MPO activity along with significantly decrease SOD and CAT activities indicating redox imbalance. Concurrent DOX+RWE treatment caused partial attenuation of redox imbalance marked by a significant reduction in MDA level and MPO activity and a significant increase in SOD and CAT activities compared to the DOX-treated rats. Data are expressed as mean ± standard deviation. *,**,*** Indicate significance *vs*. control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), #,##,### indicate significance *vs*. RWE-treated groups (#$p < 0.05$, ##$p < 0.01$, $^{###}p < 0.001$), and @,@@,@@@ indicate significance *vs*. DOX-treated groups (@$p < 0.05$, @@$p < 0.01$, @@@$p < 0.001$).

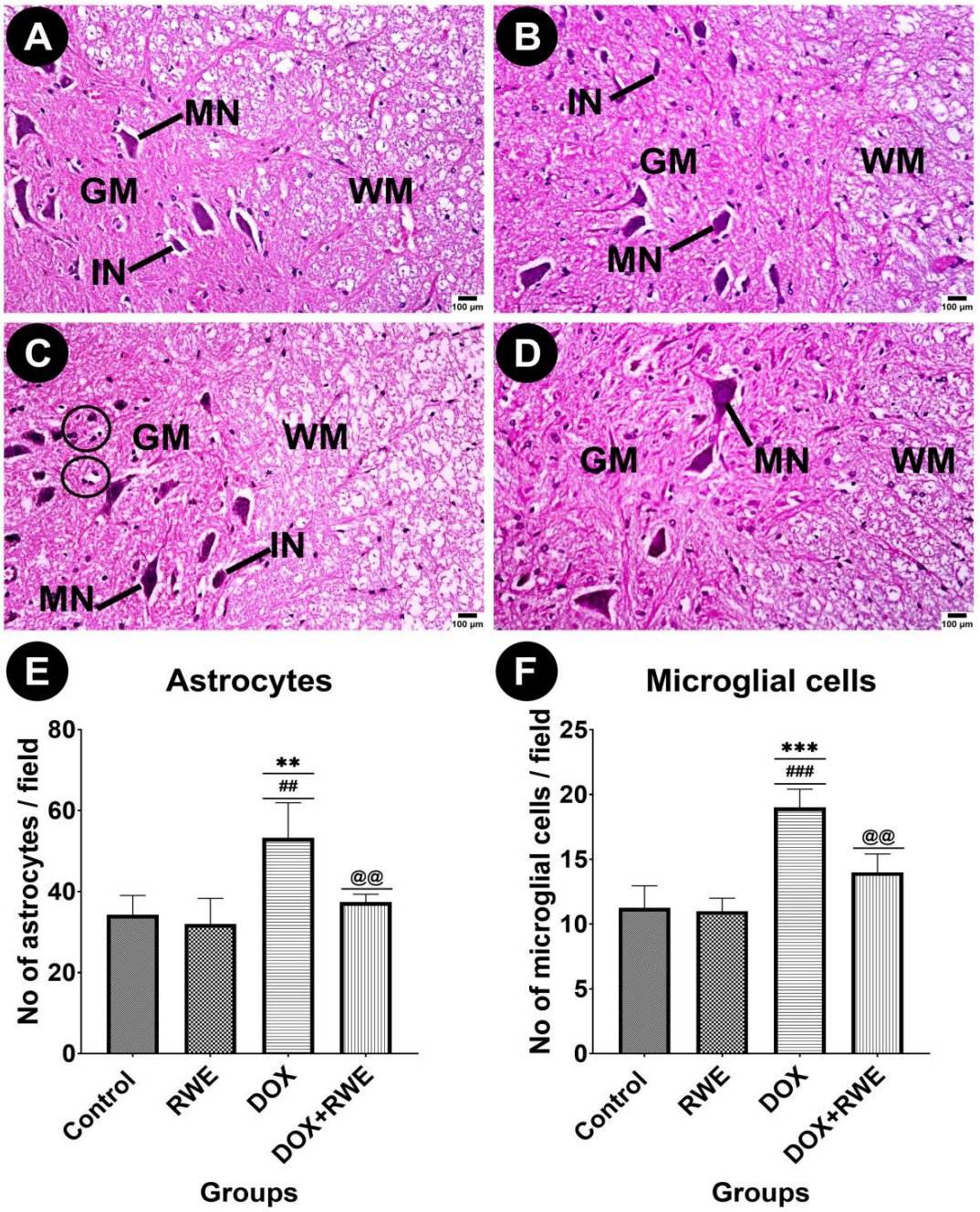

Fig. 3. Effect of DOX and RWE on spinal cord tissue structure (400X). A&B: Control and RWE-treated rat's spinal cord sections showing an outer layer of white matter (WM) and centrally located gray matter which consists of two nerve cells types multipolar neuron (MN) and interneuron (IN). C: DOX-treated rat's spinal cord section showing an increase in dispersed astrocytes (black circles). D: DOX+RWE-treated rat's spinal cord section showing regular spinal cord structure. E&F: Mean number of astrocytes and microglial cells in the spinal cord section. Data are expressed as mean ± standard deviation. **,*** Indicate significance *vs*. control (**p < 0.01, ***p < 0.001), ##,### indicate significance *vs*. RWE-treated groups (##p < 0.01, ###p < 0.001), and @@,@@@ indicate significance *vs*. DOX-treated groups (@@p < 0.01, @@@p< 0.001).

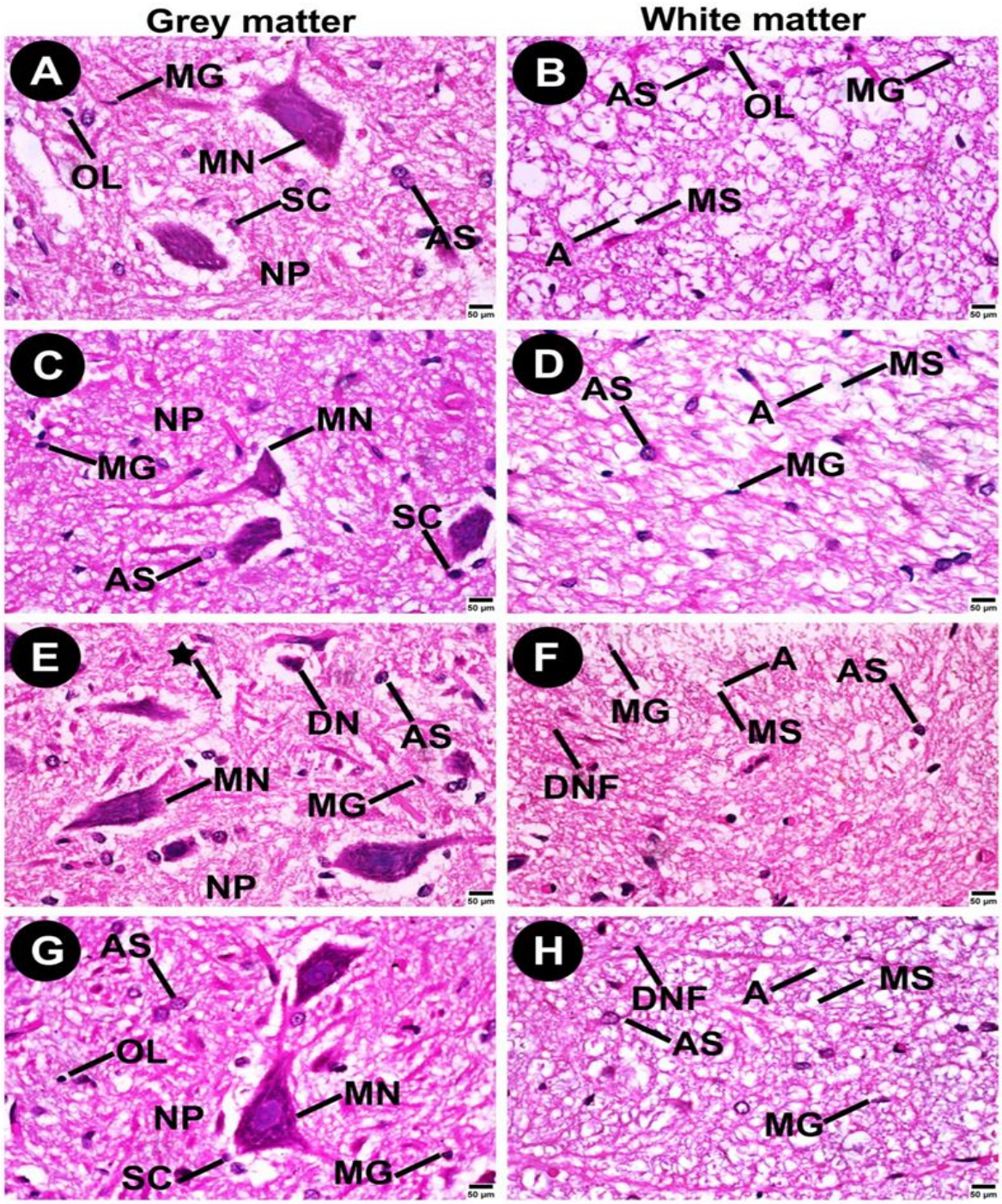

Fig. 4. Hematoxylin and eosin-stained spinal cord sections from different groups (1000X). A-D: Control and RWE-treated rat's spinal cord grey and white matter showing multipolar neurons (MN), satellite cells (SC), oligocytes (OL), astrocytes (AS), microglial cells (MG), and homogenous neuropil (NP) in grey matter. Intact nerve fiber axon (A) surrounded by a myelin sheath (MS) in white matter. E&F: DOX-treated rat spinal cord grey and white matter showing normal multipolar neurons (MN), degenerated neuron (DN), and neuropil spongiosis (star), while in white matter disrupted axon arrangement with degenerated nerve fibers (DNF). G&H: DOX+RWE-treated rat's spinal cord grey and white matter showing normal grey matter structure and homogenous pink neuropil (NP), mild degenerated nerve fibers (DNF) in between regular nerve fibers.

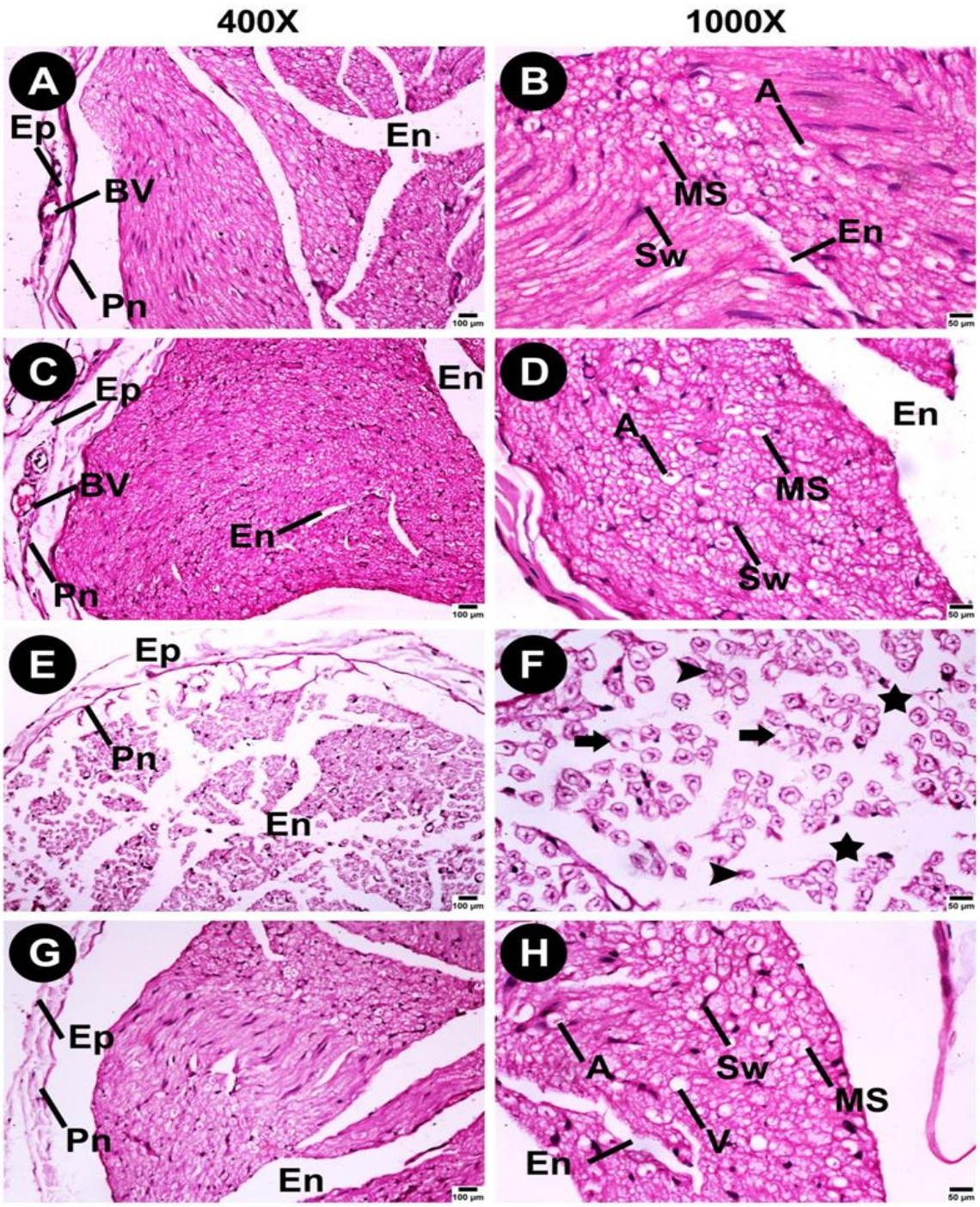

Fig. 5. Hematoxylin and eosin-stained sciatic nerve from different groups. A-D: Control and RWE-treated rats' sciatic nerve sections showing epineurium (Ep), blood vessel (BV), and thin perineurium (Pn) surrounding the closely packed nerve fibers fascicles that separated by small endoneurium (En) area. Nerve fiber fascicles consist of myelinated nerve fibers with central axoplasm surrounded by a myelin sheath (MS) and well-packed with Schwann cells (SC). E&F: DOX-treated group showing loosely packed nerve fibers fascicles with increased endoneurium (En), separation of nerve fibers with a wide area on endoneurium (Star), axoplasmic vacuolations and swollen (arrow), and demyelinated nerve fiber (Head arrows). G&H: DOX+RWE -treated group showing normal nerve fascicle arrangement with a mild increase in endoneurium (En), nerve fibers are closely packed with limited axoplasmic vacuolations (V).

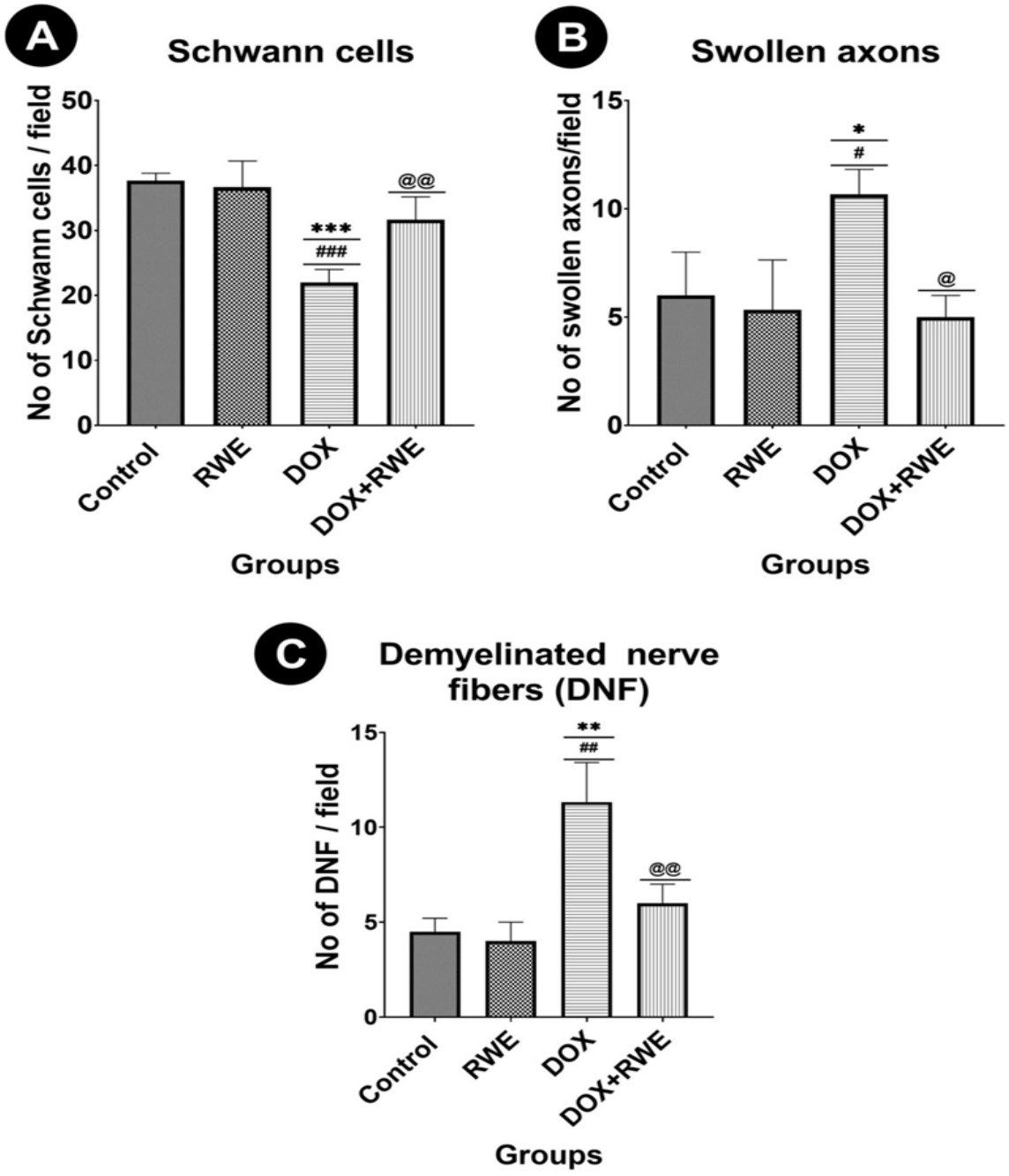

Fig. 6. Mean number of Schwann cells (A), swollen axons (B), and demyelinated fibers in the sciatic nerve (C) of different experimental groups. Data are expressed as mean ± standard deviation. *,**,*** Indicate significance *vs*. control (*p < 0.05, **p < 0.01, ***p < 0.001), #,##,### indicate significance *vs*. RWE-treated groups (#p < 0.05, ##p < 0.01, ###p < 0.001), and @@,@@@ indicate significance *vs*. DOX-treated groups (@@p < 0.01, @@@p < 0.001). DOX; Doxorubicin, RWE; Roselle water extract.

4. Discussion

The DOX, a chemotherapy drug, can cause neurotoxic effects, affecting patients' quality of life. These neurotoxic effects can manifest as peripheral neuropathy, causing symptoms such as numbness, pain in the hands and feet, and tingling (Semis et al., 2021; Selvy et al., 2022). Peripheral neuropathy, or neuropathic pain, arises from dysfunction or damage to the nervous system, originating from peripheral nerves, the spinal cord, and the brain. It is characterized by abnormal chronic pain and hypersensitivity, affecting peripheral fibers or central neurons, resulting in hyperalgesia, allodynia, spontaneous pain, and chronic inflammation (Hegazy et al., 2020; Garrido-Suárez et al., 2021). Management strategies may include painkillers, and using natural pain remedies may aid in symptom management and help improve patients' overall well-being during treatment. Roselle calyx, a traditional remedy for nervous system disorders (Edo et al., 2023; Elegbeleye et al., 2022) with antioxidant and anti-neurodegenerative properties (Efosa et al., 2023), may mitigate these effects and reduce inflammation and pain associated with peripheral neuropathy. Peripheral nerves' weakened antioxidant defenses increase vulnerability to oxidative injury, lipid peroxidation, nerve inflammation, and myelin sheath degradation, leading to nerve damage and peripheral neuropathy (Khademi et al., 2020). Oxidative stress and inflammation, are often linked to pathological conditions like chemotherapy-induced peripheral neuropathy (Balkrishna et al., 2022).

The neurotoxic activity of DOX is partially attributed to DOX-related redox imbalance and neuroinflammation (Ibrahim and Ahmed, 2021). In this study, DOX treatment (G3) resulted in elevation in serum MDA level and MPO activity and reduction in SOD and CAT activities. This imbalance contributes to spinal cord neuronal damage, reduction in Schwann cells population, and myelin sheath degradation leading to sciatic nerve injury. DOX treatment disrupts mitochondria function, leading to overproduction of ROS, redox imbalance (↑MDA, ↓SOD, ↓CAT), and neuronal damage causing the development of neurological disorders (Ali et al., 2020; Cardoso et al., 2020; Imosemi et al., 2022; Aldubayan et al., 2024). A cumulative dosage of 16mg/kg body weight of DOX increased MDA and lowered the total antioxidant capacity of plasma levels, causing peripheral nerve damage and peripheral neuropathy (Farshid et al., 2015). Yaşar et al. (2020) reported that increased levels of MPO indicate increased oxidative stress. Similarly, Imosemi et al. (2022) reported an increase in MPO, IL-1β, and TNF-α and neural degeneration in DOX-treated rats.

One of the most important contributors to the development of neuropathic pain is neuroinflammation, and several immune cells are involved in the process. In addition to the recruitment of leukocytes and neutrophils to the lesion site after nerve injury (Naveed et al., 2021), microglia and astrocytes are a key players in the development of neuropathic pain by releasing pro-inflammatory mediators (Brandolini et al., 2019). In this work, MPO plays a dual role, functioning as both an oxidative marker and an inflammatory marker. The DOX treatment (G3) caused a significant elevation in MPO activity, accompanied by a significant rise in the population of astrocytes and microglial cells. Large quantities of MPO released from recruited neutrophils cause chemotherapy-induced peripheral neuropathy (Khademi et al., 2020; Nasser et al., 2022). The activation of microglia and astrocytes due to DOX treatment can lead to peripheral inflammation, oxidative stress, neuro-inflammation, and neuronal death (Ali et al., 2020; Ongnok et al., 2021).

Kim et al. (2020) reported that chemotherapy like vincristine administration can increase MPO levels, leading to nerve fiber demyelination, Schwann cell damage, and nerve damage. DOX caused loss of dorsal root ganglion sensory neurons and Schwann cells degeneration (Farshid et al., 2015). Schwann cells damage triggers inflammation and the release of inflammatory cytokines, leading to neuropathic pain like hyperalgesia and allodynia (Khalilzadeh et al., 2018). Overall, these effects can contribute to the development and progression of peripheral neuropathy in rats undergoing DOX treatment.

Neuropathic pain is characterized by spontaneous pain, augmented response to unpleasant stimuli (hyperalgesia), and response to harmless stimuli (allodynia) (Sun and Peng, 2021). In this study, rats treated with DOX had quicker reactions to heat and cold stimuli, indicating thermal hyperalgesia, and cold allodynia. Similarly, DOX-induced free radicals can induce nerve damage leading to neuropathic pain symptoms such as cold and mechanical allodynia (Farshid et al., 2015). Chemotherapies such as paclitaxel promote demyelination of the sciatic nerve, damaging Schwann cell-formed myelin sheaths and leading to the development of neuropathic pain (Andoh et al., 2017).

Spinal cord glial cells can secrete pro-inflammatory cytokines that trigger a signal transduction cascade that releases excitatory amino acids and stimulates pain transmission (Pawar et al., 2023). Axon degeneration with a disrupted myelin sheath is a result of peripheral neuropathy, a disorder characterized by neuronal death (Serap, 2022). Overall, oxidative stress, activated spinal cord glial cells, and damaged Schwann cells are linked to the occurrence of neuropathic pain through inflammatory processes leading to nerve fibers axonal damage. This cascade of events ultimately leads to symptoms such as hyperalgesia and allodynia in DOX-treated rats.

In the present study, RWE administration alongside DOX (G4) effectively reduced MDA level and MPO activity while boosting SOD and CAT activities, indicating its antioxidant potential in mitigating oxidative stress (Shalgum et al., 2019; Mishra et al., 2022). This group (G4) showed a lower number of degenerated neurons, and demyelinated nerve fibers compared to the DOX-treated group (G3). The neuroprotective action of roselle extract is derived from its capacity to eliminate ROS, reduce lipid peroxidation, and enhance CAT levels, thus preventing damage to neurons. This effect is attributed to the extract's high content of flavonoids, phenolics, and antioxidants (Shalgum et al., 2019; Mezni et al., 2020). It also reduces MDA level and MPO activity, neutralizes iron neuron toxicity, and reduces axonal degeneration due to its flavonoids, and phenolic content (Mishra et al., 2022). Flavonoids, glycosides, and tannins in roselle extract inhibit lipid peroxidation and increases the SOD and CAT activities, preventing aluminum oxide neurotoxicity and neurodegeneration (Efosa et al., 2023). The current study showed that the antioxidant activity of RWE prevents DOX-oxidative stress and protects the spinal cord and sciatic nerve from DOX-induced neurotoxicity.

In the present study, the administration of RWE in addition to DOX reduced the neuroinflammatory effects of DOX. This was marked by attenuation of microglial cell and astrocyte activation, preservation of Schwann cells population, limiting sciatic nerve axonal degeneration, leading to preventing DOX-induced neuropathic pain. The roselle extract demonstrates anti-inflammatory properties in microglia and neuroblastoma cells by inhibiting the formation of nitric oxide (dan Neuroblastoma, 2016). Roselle extract administration ameliorates axonal damage in the spinal cord (Mishra et al., 2022). Uzar et al. (2010) reported that the neuroprotective action of caffeic acid phenethyl ester is characterized by its ability to preserve the population of Schwann cells in rats intoxicated with methotrexate, hence preventing damage to the peripheral nerves.

Flavonoids can alleviate peripheral neuropathy symptoms like thermal hyperalgesia and cold allodynia by inhibiting proinflammatory cytokine release, and axonal swelling, and preventing neuronal damage or death (Siddiqui et al., 2021). Oxidative stress reduction protects Schwann cells, preserving the axon myelin sheath and reducing mechanical allodynia (Andoh et al., 2017). These findings highlight the potential benefits of combining roselle extract with DOX in the reduction of neuroinflammation and neuropathic pain by shielding Schwann cells from DOX-oxidative damage.

Conclusion

The present study underscores the significant neurotoxic effects of DOX as a chemotherapy, particularly its impact on peripheral neuropathy and neuropathic pain. It also highlights the potential of roselle water extract (RWE) as a complementary therapy to mitigate DOX-induced neurotoxicity. RWE's antioxidant and

neuroprotective actions showed promise in protecting nerve structures and reducing neuropathic pain associated with DOX treatment. Overall, the findings suggested that roselle extract has potential therapeutic benefits for conditions involving oxidative stress and neuronal damage. Further research is needed to validate these findings for clinical applications.

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