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Nrf2/HO-1 Pathway Mediates the Protective Impact of Metrnl on Oxaliplatin-Induced Peripheral Neuropathy in Rats

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Abstract

Introduction: While oxaliplatin (OXL) is an effective cancer chemotherapy, OXL-based therapy's practical usefulness is limited by its adverse effects, which include peripheral neuropathy (PN). ***Objective***: to assess the potential underlying processes and the protective impact of metrnl in PN caused by OXL. ***Materials and Methods*:** There were three groups of thirty adult male albino rats: control, OXL and OXL+Metrnl groups (10/group). Electromyography (EMG) and nerve conduction velocity (NCV) were applied to rats, together with behavioral testing for pain and sensory abnormalities. The expression of several genes was evaluated in the sciatic nerve, including tissue MDA, (SOD), TNF-α, interleukin (IL)-6, IL-10, caspase-3, BDNF, nuclear factor E2-related factor 2 (Nrf2), and homo-oxygenase 1 (HO-1). Additionally, histopathological evaluations of the gastrocnemius and sciatic nerve were carried out. ***Results:*** The OXL group exhibited mechanical and cold allodynia, as well as significantly higher levels of MDA, TNF-α, IL-6, and caspase-3 than the control group. It also significantly reduced NCV, EMG recordings, IL-10, as well as down-regulating BDNF, Nrf2 and HO-1 gene expression in the sciatic nerve. OXL-induced PN changes were significantly ameliorated in the OXL+Metrnl groups. ***Conclusion***: Metrnl has valuable protective effects on OXL-induced PN via anti-oxidant, anti-inflammatory, inhibition of apoptosis and neurotrophic effects in addition to up-regulation of Nrf2 and HO-1 gene expressions.

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Introduction

Peripheral neuropathy (PN) is induced in up to 80% of individuals by chemotherapy medications. Even though oxaliplatin (OXL) is frequently used to treat colorectal cancer (CRC), it causes significant peripheral neuropathy (PN), which restricts its clinical use because it lowers quality of life and may require reducing or stopping the medication. As a result, cancer patients' therapeutic options are reduced, and their quality-of-life declines. We don't have enough research to prevent neuropathic pain brought on by chemotherapy. [1]. Themodification of voltage-gated Na + channels are the primary mechanism linked to OXL-induced PN with nuclear DNA damage, mitochondrial damage, oxidative stress from overload, glial activation, oxidative stress from mitochondrial damage, axonal degeneration, neuronal apoptosis, and neuroinflammation.[1]. Itis well recognized that brain-derived neurotrophic factor (BDNF) plays main role in neurogenesis, learning, and synaptic plasticity. It has been determined that BDNF controls neuronal survival.[2]. OXL in brain tissue inhibits BDNF expression, resulting in neurodegeneration and neuroinflammation [3]. The antioxidant response element (ARE) is the mechanism by which the principal regulator of the antioxidant response, nuclear factor E2-related factor 2 (Nrf2), regulates numerous genes that encode antioxidant proteins. [4]. The oxidant-responsive transcription factor Nrf2 promotes cytoprotection against oxidative stress by modulating the expression of HO-1 [5].

Secreted proteins have the potential to serve as biomarkers and as interventions for disorder and aging. They also play significant functions in physiological and pathological mechanisms. Metrnl is a new protein that is released. Its expression is mostly found in the brain, though it is distributed throughout the body more widely. This protein is involved in insulin sensitivity, white adipose browning, and brain development. Considering its expression and unique roles—which pertain to its adipokine activity, neurotrophic effect, and potential cytokine action, respectively. The range of Metrnl activities and mechanism is yet to be identified. The development of novel treatment strategies for a variety of maladies may be facilitated by the neurotrophic activity and advantageous metabolic impact associated with that protein. To use this recently identified protein as a therapeutic target or drug, however, further research must be done to comprehend its range of functions in both health and sickness, including the critical mechanisms at play.[6]

This prompted us to carry out the current research to assess the beneficial effect of Metrnl on OXL-induced PN for the 1st time. Additionally, the potential mechanisms that underlie such impacts were examined.

Methods

Animals

Under the guidelines set forth by the Faculty of Medicine at Menoufia University's Animal Experimentation Ethics Committee with IRB NO (10/2024Bio17). Thirty adult male Wister rats were used in this study and weighed between 150 and 180 g. The rats were housed at temperatures ranging from 20 to 24 degrees Celsius. They also had free gain access to potable water and standard rat food. Prior to commencement of the

experiments, the rats were given ten days to acclimate.

Study groups

(1) Control group: Rats were received intraperitoneal (i.p.) injection of 1 mL glucose 5% daily for two weeks and 1 mL phosphate buffer saline (PBS) (Sigma-Aldrich Company, St. Louis, USA) intravenously daily for two weeks.

(2) Oxaliplatin induced peripheral neuropathy (OXL) group: Rats were received OXL (Hikma Pharmaceuticals Plc company, Egypt) with a dose of 2.4 mg/kg/day mixed with 1 ml glucose 5% i.p. daily for 5 consecutive days every week for two weeks. [7].

(3) Oxaliplatin induced peripheral neuropathy Metrnl treated (OXL+Metrnl) group: Rats were given OXL 2.4 mg/kg/day i.p. daily for two weeks and Metrnl (Adipogen, San Diego, CA, USA) intravenously (2 μg/rat/day) dissolved in 1 mL of PBS daily for two weeks beginning on day 1 [8].

Electromyography (EMG) and behavioral assessment were doneat the end of the experiment, the rats were sacrificed by cervical elongation and dislocation after being anesthetized, and sciatic nerves from the ischiatic notch to the popliteal fossa on both sides were dissected to measure nerve conduction velocity. Furthermore, the gastrocnemius muscle was dissected. For the biochemical tests we used the left sciatic nerves, whereas for the histopathological studies we used the right sciatic nerve and the gastrocnemius muscle.

Behavioral assessment

On day 14 of the experiment, a behavioral assessment of pain and sensory disturbances was completed.

1. Paintbrush test

The mechanical dynamic allodynia was assessed utilizing this behavioral test. Mechanical allodynia is a painful condition that is caused by mild stimulus, such as light touch. Plastic cages were utilized to house the rats on a wire mesh-covered floor. The plantar region of the hind paw was rubbed with a smooth paintbrush five times, at five-second intervals, from heel to toe. It was documented how many withdrawals there were (0 to 5). Since normal rats do not react to this type of stimulus, the withdrawal reflex has been deemed as a marker of mechanical dynamic allodynia. The exam was administered three times, with a five-minute pause between each. Each rat's total score was recorded, with a minimum of zero and a maximum of 15 [9].

2-Acetone test

Cold allodynia was assessed using the acetone spray method. The rats were placed in plastic wire-mesh cages, and 100 microliters of acetone was sprayed onto the plantar areas of their hind paws. The rat's response was recorded for 20 seconds and then rated on a four-point scale: 0 for no response, 1 for rapid paw withdrawal or flicking, 2 for prolonged paw withdrawal or repeated flicking, and 3 for repeated paw flicking in association with licking. Acetone was administered to the hind paw three times, each separated by five minutes. Each rat's three repetition scores were added to yield a single score with a minimum of 0 and a maximum of 9. [10].

3. Tail flick latency test

Rats' thermal nociceptive perception was assessed using the Harvard Apparatus (USA) tail flick apparatus. Heating radiation was applied to the

rats' tails at a distance of 3 cm from the tip user after they had been gently restrained. It was determined the duration it required the rat to flick its tail (tail-flick latency). To avoid tissue injury, the heat stimulus was discontinued after 10 seconds (cut-off delay). [11]

Electrophysiological evaluation

1- EMG

The Biopack MP100 acquisition equipment (Inc., Goleta, California, USA) was used to measure EMG recordings in vivo and detect muscle electrical disturbances [12]. In rats sedated with thiopental sodium (50 mg/kg, i.p), two thin concentric needle electrodes were placed into the gastrocnemius muscle to record EMG [13]. Signal inputs were made via a percutaneous puncture in the gastrocnemius muscle using two shielded electrodes (LEAD110S/EL503 or EL508S) and a ground electrode in the rat tail. Three to four minutes were spent monitoring EMG activity after electrode placement. After the test, rats were fully recovered and returned to their enclosures. The combined EMG data's average power and frequency of electrical activity were utilized to qualitatively measure myotonic-like activity.

2- NCV

NCV was measured using the Biopack Acknowledge mp 150 system (BIOPAC Systems Inc., Santa Barbara, USA). Exsanguination and cervical dislocation were used to sacrifice the rats. After being divided from the spinal emergence to the knee, the left sciatic nerves were preserved in regular Ringer's solution. The Biopac mp150 data acquisition system was used to stimulate and record nerves. The acrylic nerve stimulation holder measured 8 by 4.5 by 2.5 cm. Ringer's solution was added to the chamber, and a 15–35 mm segment of nerve was inserted so that it made good electrical contact with the electrodes used for stimulation and measurement. A stimulus was administered at intensity approximately 1.25 times higher than the compound action potential (CAP) maximal height. The nerve conduction velocity (NCV) was estimated by dividing the distance between the stimulating and recording electrodes by the duration that transpired between the onset of the stimulation and the point at which 50% of the rise in the CAP component was reached.

Tissue homogenate preparation

Using a tissue homogenizer (MPW120, MPW Medical Instruments, China), specimens from the left sciatic nerves were weighted and homogenized individually at the conclusion of the study period. After centrifuging the crude tissue homogenate in an ice-cold centrifuge for 15 minutes at 10,000 rpm, the supernatant was collected and kept at -80°C for subsequent biochemical testing.

Biochemical analysis

Nerve interleukin 10 (IL-10), IL-6, TNF-α, and Caspase 3 levels were evaluated using ELISA kits for rats. Follow the manufacturer's instructions to use IL-10 (ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA), TNF-α (ERT2010-1, Assaypro LLC, Saint Charles, Missouri, USA), IL-6 (ab100772, Abcam, Cambridge, UK), and caspase 3 (MBS018987, MyBioSource, Sandiego, CA, USA). Colorimetric kits(Biodiagnostic Company, Dokki, Giza, Egypt) were used to measure nerve malondialdehyde (MDA) and superoxide dismutase (SOD) levels.

Gene expression analysis

Gene expression quantification using reversetranscriptase polymerase chain reaction

technique, (RT-PCR). The relative mRNA levels of the BDNF, Nrf2, and HO-1 genes in the sciatic nerve were examined using RT-PCR. Following the manufacturer's directions, total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Up to use, the isolated RNA was kept at -80 C. By using a nanophotometer N60 to measure the absorbance at 260 nm (A260), RNA purity and amount were ascertained.ThermoScript RT reagent kits (Invitrogen) were used for the complementary DNA (cDNA) synthesis (reverse transcription phase) in the initial PCR process. After that, SYBR Green Mix kits (Stratagene-USA) were used to amplify cDNA. Cycle threshold, or Ct, values were obtained for each amplification curve. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

GAPDH primers sequence was:

(1) Forward primer: 5 -GTCTCCTCTGACTTCAACAGCG-3

(1) (2) Reverse Primer: 5 -ACCACCCTGTTGCTGTAGCCAA-3.

The 2.0.1 version of the 7500 ABI PRISM (AppliedBiosystems-USA) was utilized to analyze data. BDNF, Nrf2 and HO-1 relative quantification and gene expression were done using comparativeΔΔCtapproach.[14]

The following primers were used for the BDNF gene:

(2) forward primer: 5-AAACATCCGAGGACAAGGTG-3

(3) reverse primer :5-AGAAGAGGAGGCTCCAAAGG-3.[14]

The following primers were used for the Nrf2 gene:

(1) Forward primer: 5-GGTTGCCCACATTCCCAAATC-3

(2) Reverse primer: 5-CAAGTGACTGAAACGTAGCCG-3[14]

The following primers were used for the HO-1 gene:

(1) Forward primer: 5-CCATAGGCTCCTTCCTCCTTTC-3

(2) Reverse primer: 5-GGCCTTCTTTCTAGAGAGGGAATT-3[14]

Histopathological assessment*:*

Tissue samples were treated with formalin solutions, alcohol, and xylol before embedding in paraffin blocks. Materials were then sliced to 5 μm using a microtome and stained with H&E. Deparaffinized slides are rehydrated with deionized water. Stain for 10 minutes with 0.04% toluidine blue. Rinse carefully with deionized water three times (30 seconds each). These samples were histopathologically evaluated under a light microscope. [15]

Statistical analysis.

SPSS Statistical Package for Social Science software, version 16 (SPSS, Inc., USA), was used to tabulate and analyze the data. To ascertain whether the data have a normal distribution, apply the Shapiro test. The statistical information was presented as mean ± standard deviation (X±SD). A post hoc Tukey test was conducted after a one-way analysis of variance (ANOVA) to ascertain the significance of the differences between the groups. A statistically significant value ($p<0.05$) was defined as a probability value less than 0.05.

Results

Interpretation of behavioral tests

The paintbrush test score raised substantially in the OXL group compared to control (11.8333 ± 0.98319 vs 2.0000 ± 0.89443, *P*<0.05). Although the OXL+Metrnl showed a substantial reduction compared to OXL group (5.6667±0.81650) (*P*<0.05), it remained substantially higher than the control.(Fig.1.A)

The acetone test score was considerably elevated in the OXL group compared to control (7.0000±0.89443 vs 0.3333±0.51640, *P*<0.05). However, the OXL+Metrnl showed a considerable drop (3.3333 ± 0.51640) compared to the OXL group (*P*<0.05) but remained much higher than the control. (Fig.1.B)

The tail flickering test resulted in a substantial decrease in the OXL group compared to control (2.4833 ± 0.25626 s vs 7.3500 ± 0.19748 s, P<0.05). However, the OXL+Metrnl showed a considerable increase relative to the OXL group (5.2500 ± 0.23452 s) (P<0.05) but was still much lower than the control group. (Fig.1.C)

Interpretation of electrophysiological assessment tests:

For both mean frequency and power of EMG, they were both significantly decreased in OXL group when compared to control (432.33±5.50151 mv/s vs 638.33±11.03932 mv/s, 0.2550±0.03391 mv vs 0.9433±0.04131 mv respectively) (*P*<0.05). However, in OXL+Metrnl group, they were significantly increased compared to OXL group (521.17±8.42417mm/s and 0.6317±0.02927 mv) (*P*<0.05), but they still significantly less than control (Fig.2. E, F).

The nerve conduction velocity was considerably lower in the OXL group compared to the control (38.6667±2.65832 mm/s vs 91.5833±3.56955 mm/s, P<0.05). Although the OXL+Metrnl group showed a substantial rise compared to the OXL group (63.7833 ±1.05909 mm/s) (P<0.05), it remained significantly lower than the control. (Fig.2.G).

Oxidative stress markers results:

Nerve MDA were considerably higher in OXL compared to control (20.3167 ± 0.58452 nmol/gm. tissue vs 7.4000 ± 0.37417 nmol/gm. tissue, P<0.05). OXL+Metrnl showed a considerable drop (14.4167±0.26394 nmol/gm. tissue) compared to OXL group (P<0.05) but remained much higher than control. (Fig.3.A)

Nerve SOD was considerably lower in OXL group compared to control (1.1667 ± 0.08641 u/gm tissue vs 4.5033 ± 0.28994 u/gm tissue, P<0.05). OXL+Metrnl group showed a considerable rise relative to OXL group (2.6017±.07521 u/gm tissue) (P<0.05), but lower than control. (Fig.3.B).

Inflammatory mediators` results:

Both nerve TNF α and nerve IL 6 levels were substantially elevated in OXL group compared to control (60.6667±1.21106ng/ml vs 22.2500±1.72482 ng/ml, 148.83±2.13698pg/ml vs 76.4167±1.74404 pg/ml, correspondingly) (P<0.05). However, in OXL+Metrnl group, they were dramatically lowered compared to the OXL group (41.8333±2.16025 ng/ml and 96.0000±2.82843 pg/ml subsequently) (P<0.05), but still significantly greater than control . (Fig.4.A, B).

Nerve IL10 levels considerably dropped in OXL group as opposed to control (5.7983±.25926 ng/ml vs 17.0500±0.64730ng/ml) (P<0.05). The OXL+Metrnl group showed a considerable rise relative to OXL group (11.2167±0.46224ng/ml) (P<0.05) but remained much lower than the control. (Fig.4.C).

Nerve caspase 3

Tissue caspase 3 levels were considerably higher in OXL group compared to the control (63.0000 ± 2.36643 ng/ml vs 26.3333 ± 2.80476 ng/ml, P<0.05). The OXL+Metrnl group showed a substantial reduction (35.0833±3.66629ng/ml) compared to the OXL group (P<0.05) but remained substantially higher than control. (Fig.5).

Gene expression of BDNF, Nrf2 and HO-1 results:

The three genes, BDNF, Nrf2, and HO-1, was considerably lower in OXL group compared to control group (0.3750±0.03271, 0.2800±0.02098, and 0.3650±0.01871, respectively) (P<0.05). The OXL+Metrnl showed considerable upregulation compared to the OXL group (0.7550±0.03017, 0.6150±0.02881, and 0.7333±0.02160, respectively) (P<0.05), but remained significantly downregulated compared to control. (Fg. 6.A, B, C).

Histopathological assessment results:

1- Gastrocnemius muscle evaluation

Normal muscle fibers are polygonal in shape and contain several nuclei underneath the sarcolemma. Muscle fibers are surrounded by endomysium, which are thin connective tissues. The perimysium separates muscle fascicles. The OXL group had randomly distributed degenerated muscle fibers. Inflammatory cells invaded necrotic fibers. The presence of internalized nuclei with varying fiber sizes indicated myofiber damage. The OXL+Metrnl group showed improvement in cellular infiltration and muscle injury but remained deteriorated compared to the control, cell infiltration was decreased in comparison with control. (Fig. 7).

2- Sciatic nerve evaluation

A section of the control sciatic nerve has epineurium encircling it, tightly packed myelinated nerve fibers of varied diameters, and little endoneurium between them. Each nerve fiber is made up of a core nerve axon wrapped by a myelin sheath. The OXL group discovered notable features of myelin sheath and axon degeneration, as demonstrated by vacuolation and cavitation in the sciatic nerve trunk, as well as inflammation signs such as cellular infiltration. Metrnl therapy lowered myelin sheath and axon degeneration, as well as vacuolation and cavitation. Cell infiltration was decreased in comparison with control. (Fig.8, 9)

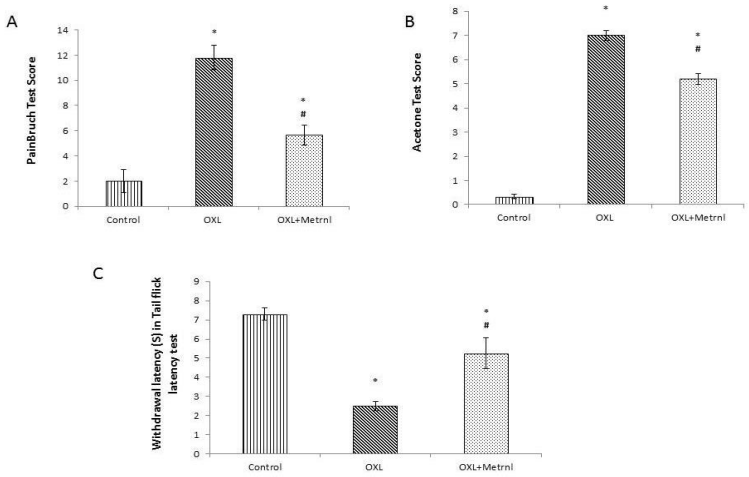


Fig 1. Metrnl impact on A) PainBrush test score, B) Acetone test score and C) Withdrawal latency in Tail flick latency test (S) in all studied groups. * Significant compared to control, #significant compared to OXL group.

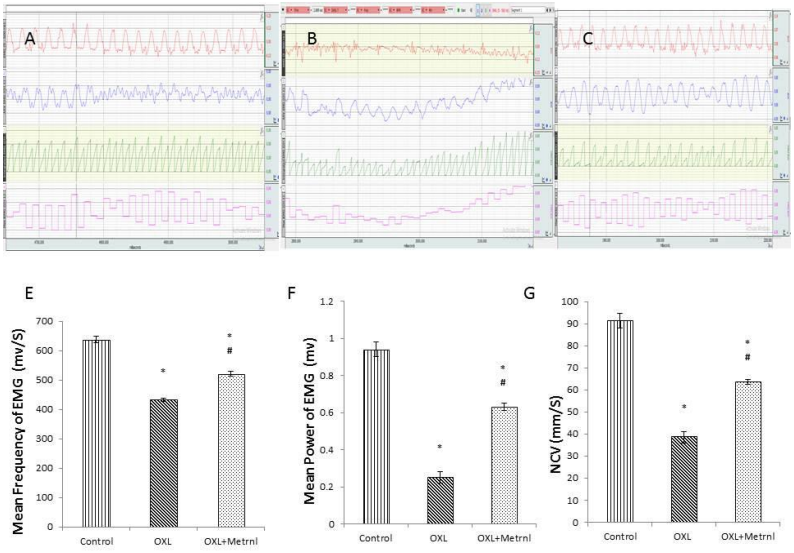


Fig 2. Metrnl impact on Electrophysiological record in A) Control, B) OXL and C) OXL+Metrnl E) Mean frequency of EMG (mv/S) F) Mean power of EMG (mv). G) NCV (mm/S) * Significant compared to control, # significant compared to OXL group.

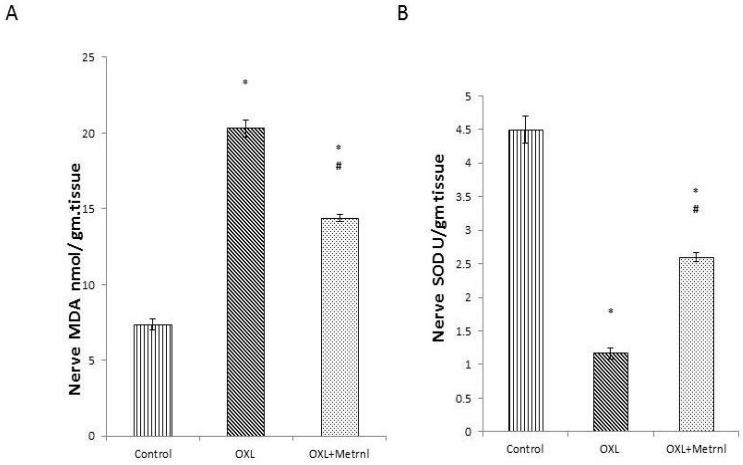


Fig 3. Metrnl impact on A) Nerve MDA (nmol/gm.tissue), B) Nerve SOD (U/gm tissue). * Significant compared to control, # significant compared to OXL group

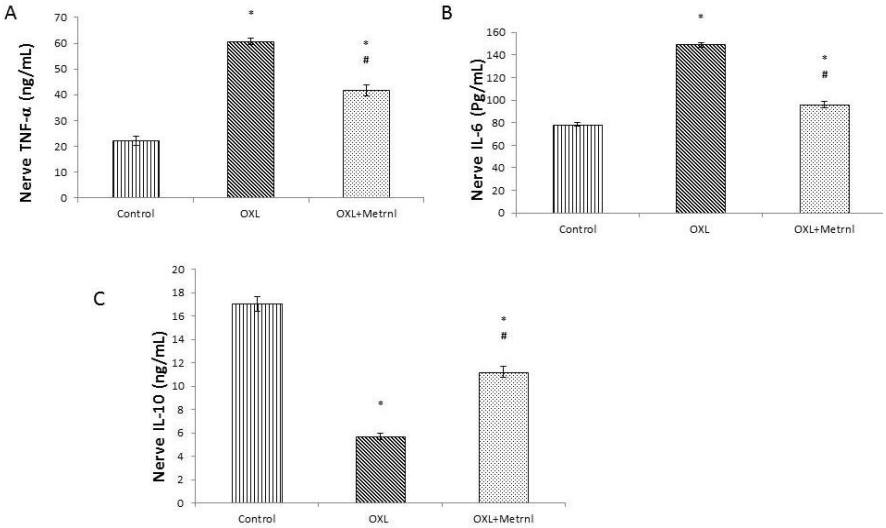


Fig 4. Metrnl impact on A) Nerve TNF-α (ngα () , B) Nerve IL-6 (Pg/mL), c) Nerve IL-10 (ng/mL) . * Significant compared to control, # significant compared to OXL group

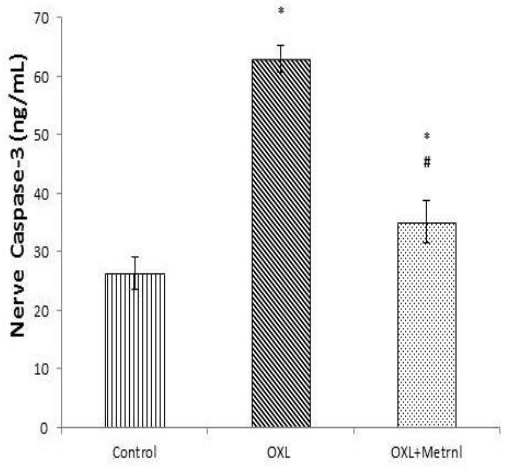


Fig 5. Metrnl impact on Nerve Caspase-3 (ng/mL). *Significant compared to control, # significant compared to OXL group

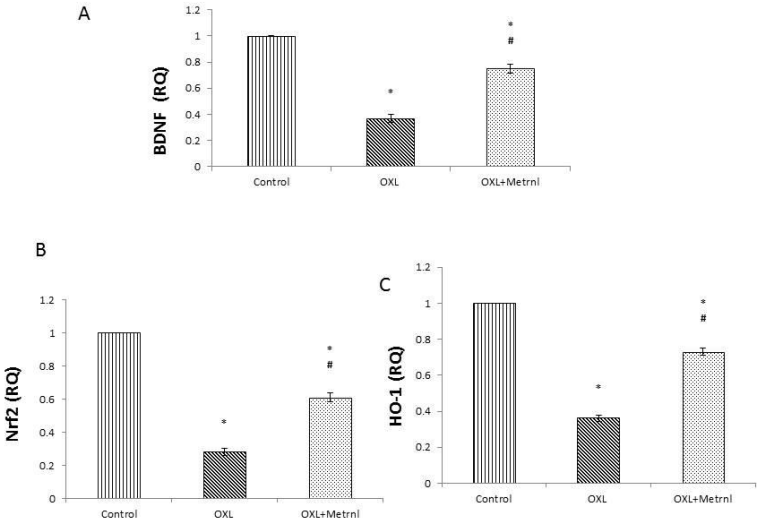
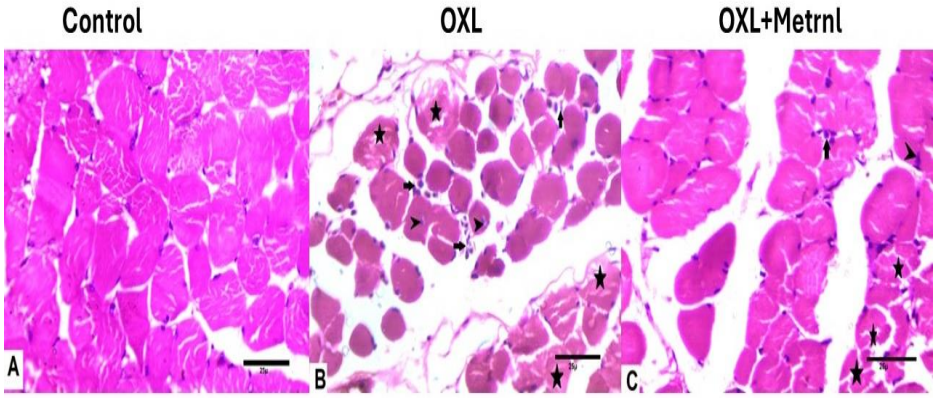


Fig 6. Metrnl impact on sciatic nerve gene expression of A) BDNF, B) Nrf2 andC) HO-1. * Significant compared to control, # significant compared to OXL group



H&E-stained sections of the gastrocnemius muscle revealed: A) normal muscle fibers. B) OXL group. C) The OXL+Metrnl group. Degeneration of muscle fibers (Astrex), inflammatory cellular infiltration in the endomysium (arrow), and centralized muscle fiber nuclei (arrowhead). (Hx&E X400).

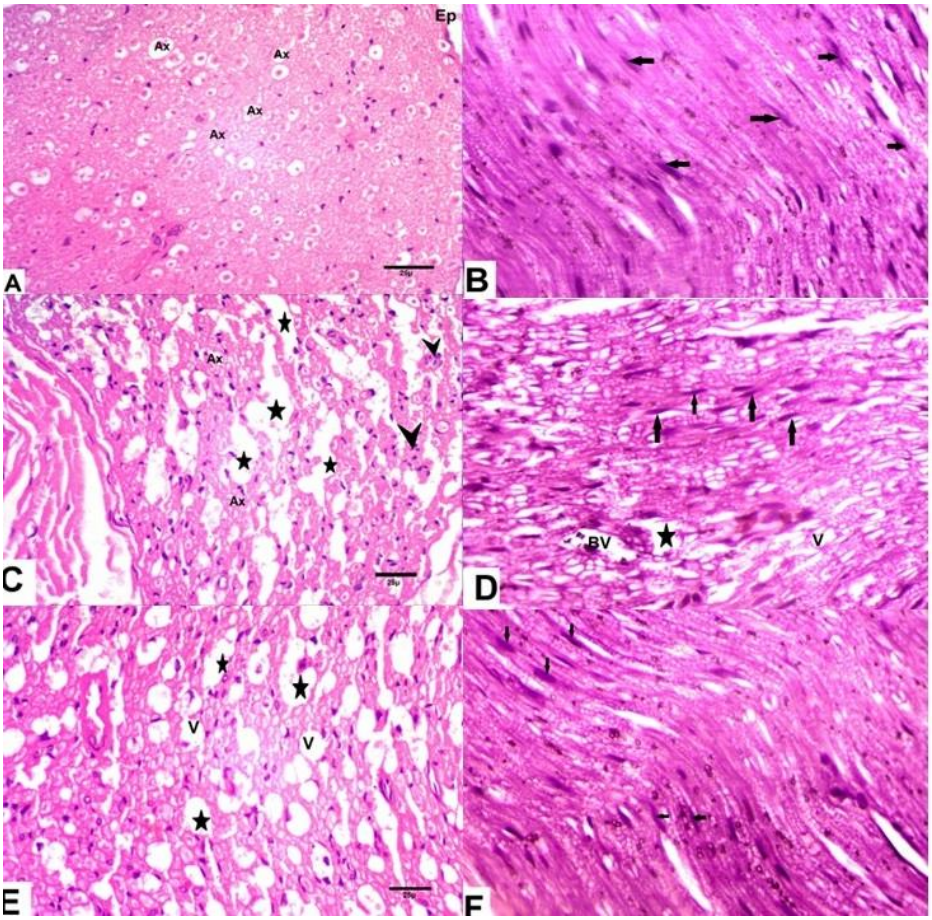


Fig. 8A photomicrograph of a transverse section of the sciatic nerve reveals A and B) the control nerve surrounded by the epineurium (Ep) and myelinated nerve fibers of varying sizes, each consisting of a central nerve axon (Ax) surrounded by myelin sheaths.Schwann cells have elongated nuclei(arrow). The OXL group contains less myelinated nerve fibers with core axon (Ax), myelin sheath, and axon degeneration as seen by vacuolation (V), cavitation (Star), cellular infiltration (arrowhead) and dilated blood vessels (BV). (E, F) OXL+Metrnl group exhibits less cavitation and vacuolation. (Hx&E X400)

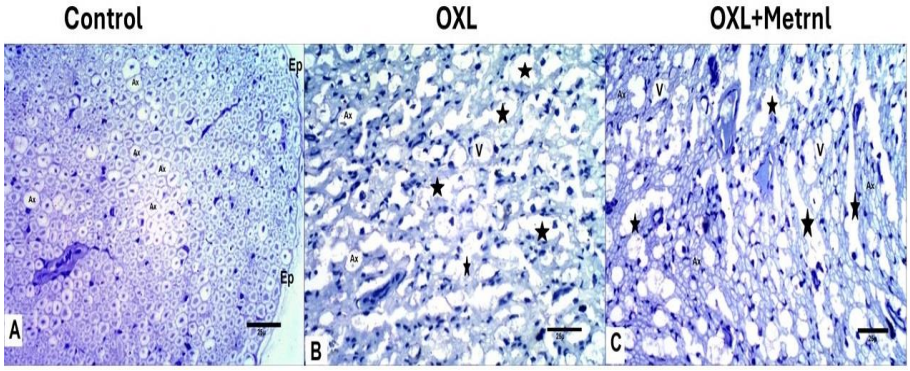


Fig .9 Toluidine blue staining of sciatic nerve cross-sections in the (a) control, (b) OXL, and (c) OXL+Metrnlgroups shows normal myelinated axons (Ax) in the control group, whereas treated groups display vacuolation (V) and cavitation (star). (TB X400)

Discussion

Oxaloplatin's clinical application is limited due to the PN it causes. Secreted proteins serve as biomarkers and therapy targets for a variety of disorders. They also have crucial physiological and pathological functions. Metrnl is distributed more widely throughout the body. The goal of this study was to look into Metrnl's ability to protect against PN induced by OXL, as well as to identify any relevant mechanisms.

This study found that OXL caused behavioral changes such as enhanced sensitivity to heat and a

stronger response to mechanical stimulation and low temperatures. To do this, we used the paintbrush, acetone, and tail flick latency experiments. In this study, the group that got OXL had a considerably greater nociceptive score, as well as mechanical dynamic and cold allodynia. These results are consistent with earlier studies. The user's text reads "[16]." The NCV of the sciatic nerve, the EMG frequency of the OXL group, and the power of the gastrocnemius muscle all decreased significantly, which is consistent with earlier findings [17]. Sensory nerve conduction anomalies are among the early symptoms of oxaliplatin-induced nerve damage [17]. Furthermore, Cavaletti et al. [18] showed that chronic oxaliplatin administration in rats resulted in decreased peripheral nerve conduction velocity (NCV) and neuronal degeneration in the dorsal root ganglia (DRG).

Metrnl lessened the behavioral alterations brought on by OXL. Furthermore, when compared to the OXL group, Metrnl dramatically enhanced the NCV of the sciatic nerve, the frequency and power ofthe gastrocnemius muscle's EMG. These findings support the finding that Metrnl has a neuroprotective effect[19]. The present data corroborate the analgesic potential of Metrnl during OXL-induced PN, improving behavioral and electrophysiological parameters.

The primary causes of OXL-induced PN are ion channel disruption, neuronal inflammation, neuronal injury, and oxidative stress. [20]. The current investigation found that the OXL group had significantly lower levels of the anti-inflammatory marker IL-10 and significantly higher levels of the pro-inflammatory cytokines leading to neuroinflammation. This is consistent with prior investigations [21]. OXL activates NF-κB and increases the production of cytokines that cause inflammation. Inflammatory markers are employed at the region of the nerve lesion in neuropathic pain, which causes the pain to develop. [21]

Mernl significantly reduced the inflammatory process brought on by OXLby inhibiting the generation of pro-inflammatory cytokines, the Metrnl protein decreased inflammation and prevented kidney damage in a model of sepsis in mice; moreover, METRNL decreased inflammation through pathways that were dependent on PPARδ. [22]. Metrnl reduces inflammation by decreasing the activation and nuclear translocation of NF-κB. [23].

Oxidative damage may be linked to chemotherapy-induced peripheral neuropathy. Our data demonstrated this by showing a considerable rise in MDA and fall in SOD levels. It is believed that platinum deposits in tissues contribute to the equivalent increase in ROS generation, which in turn causes rats exposed to OXL to exhibit increased mechanical and thermal sensitivity.[24]. According to reports, using OXL results in mitochondrial dysfunction and peroxidation of cell membranes in the neurological system[25]. A rise in MDA levels damages cells severely and compromises their ability to form their membranes, which can lead to the cells entering the apoptotic process. [26].

When compared to the OXL group, metrnl therapy significantly improved oxidative stress markers. Metrnl's antioxidant activity was in line with [27]. Metrnl increases glucose metabolism and lipid oxidation in skeletal muscle via autocrine/paracrine signaling pathways [28], and it

exploits autocrine processes to fight doxorubicin-induced oxidative stress.[29]

In agreement with the results of Ta et al, our findings revealed that the OXL group had higher caspase 3 levels. [30]. Caspase-3, the principal caspase, regulates several other caspases, including proteins from the Bcl-2 family. [31]. According to research, OXL induces mitochondrial dysfunction, and the sciatic nerve's increased amounts of proapoptotic proteins such as caspase-3, Bax, and cytochrome C result in caspase-dependent neuronal apoptosis. [32].

Metrnl inhibited OXL-induced apoptosis. Metrnl's anti-apoptotic abilities have already been reported by [33]. Hu et al. found that Metrnl stimulates the cAMP/PKA/SIRT1 pathway in doxorubicin-induced cardiotoxicity, providing resistance to myocardial oxidative stress and apoptosis with increased BCL-2 protein and decreased BAX and caspase-3 levels. [34]

BDNF regulates neuronal survival and regeneration. It has the ability to profoundly influence how the central and peripheral nervous systems regulate and promote neurogenesis. [35]. According to reports, BDNF promotes the inflammatory response and can regulate it. [36] A prior study found that chemotherapeutic drugs reduce BDNF levels, resulting in neuroinflammation and neurodegeneration. [35] coincides with our findings.

Metrnl has a neurotrophic factor role. According to Jorgensen et al. [37], Metrnl has a neurotrophic effect on spiral ganglion neurons' neurite development and survival. Because Metrnl deficiency markedly increased cognitive impairment and decreased BDNF levels in the hippocampus, it is possible that Metrnl regulates cognitive dysfunction in D galactose-induced aging mice. [38]

The transcription factor Nrf2 is in charge of regulating redox equilibrium inside cells and increasing antioxidant capacity. [39] It has been proven that HO1, the inducible version of heme oxygenase, is the primary rate-limiting enzyme in heme degradation processes and a key effector of Nrf2-dependent cellular responses. [40]. OXL dramatically lowered the expression of the Nrf2 and HO-1 genes, which was consistent with previous studies. [41]

Metrnl up-regulated Nrf2 and HO-1 gene expression, as revealed by Hu et al., 2020, who discovered that Metrnl restored Nrf2 protein levels after DOX treatment, while also preserving downstream HO-1 and SOD2 protein levels.[34]

Our histopathological findings revealed that OXL induced inflammation and skeletal muscle degeneration. Other researchers validated similar findings, demonstrating that OXL therapy caused mice to gain less weight and muscle mass in the gastrocnemius, but also increased myopathy. [42]. Further studies demonstrated that OXL therapy can directly affect skeletal muscle balance and stability, resulting in muscle mass loss. [43]. The considerable increase in STAT3 phosphorylation in the gastrocnemius muscles explains these findings and linked OXL oxidative stress to mitochondrial-mediated cell damage in skeletal muscle. OXL enters the mitochondria, potentially increasing mitochondrial reactive oxygen species (mtROS) production [42]. Metrnl reduces inflammation and promotes recovery in skeletal muscle injuries. It also stimulates the creation of growth factors by macrophages, which aid in muscle regeneration.[23] .

Our findings of OXL-induced peripheral neuropathy were supported by histological results of the sciatic nerve in the OXL group. These findings are consistent with previous data indicating that OXL causes demyelination and degeneration of sciatic nerve fibers [44]. However, Metrnl decreased OXL-induced myopathy and peripheral neuropathy. Metrnl is a neurotrophic factor that nourishes neurons and performs a critical role in neuronal growth, repair, and rejuvenation [45].

Conclusion

Metrnl administration improved OXL-induced peripheral neuropathy by reducing changes in behavior, electrophysiology, and histopathology. Metrnl exerts a protective impact by inhibiting pro-inflammatory cytokines, reducing oxidative stress, inhibiting apoptosis, promoting neurotrophic effects through increased expression of BDNF, and up-regulating Nrf2/HO-1 genes. The results indicate that Metrnl could potentially be a successful interventionfor preventing OXL-induced peripheral neuropathy in a rat model. Further studied are needed to assess the effectiveness of metrnl on peripheral neuropathy and elucidate the underlying mechanisms. These findings support the need for further evaluation of this technique in a larger preclinical investigation.

References

1. **Kang L, Tian Y, Xu S, Chen H**. Oxaliplatin-induced peripheral neuropathy: clinical features, mechanisms, prevention and treatment. Journal of Neurology;268(9):3269-82, 2021
2. **Choi, D. H., Lee, K. H., & Lee, J.** Effect of exercise-induced neurogenesis on cognitive function deficit in a rat model of vascular dementia. *Molecular medicine reports*, *13*(4), 2981-2990, 2016
3. **Celik, H., Kucukler, S., Ozdemir, S., Comakli, S., Gur, C., Kandemir, F. M., &Yardim, A.** Lycopene protects against central and peripheral neuropathy by inhibiting oxaliplatin-induced ATF-6 pathway, apoptosis, inflammation and oxidative stress in brains and sciatic tissues of rats. *Neurotoxicology*, *80*, 29-40, 2020.
4. **Khodir, S., Alafify, A., Omar, E., & Al-Gholam, M.** Protective potential of ginseng and/or coenzyme Q10 on doxorubicin-induced testicular and hepatic toxicity in rats. *Open Access Macedonian Journal of Medical Sciences*, *9*(A), 993-1005, 2021.
5. **Luo Y, Shang P, Li D. Luteolin**: a flavonoid that has multiple cardio-protective effects and its molecular mechanisms. Front Pharmacol;8:692, 2017
6. **Zheng, S. L., Li, Z. Y., Song, J., Liu, J. M., & Miao, C. Y. Metrnl**: a secreted protein with new emerging functions. *Acta pharmacologicasinica*, *37*(5), 571-579, 2016.
7. **Resta F, Micheli L, Laurino A, Spinelli V, Mello T, Sartiani L, et al.** Selective HCN1 block as a strategy to control oxaliplatin-induced neuropathy. Neuropharmacology; 131:403-13, 2018

8. **Jung TW, Lee SH, Kim H-C, et al.** METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice. Experimental & molecular medicine.;50(9):1-11, 2018
9. **Vashistha B, Sharma A, Jain V.** Ameliorative potential of ferulic acid in vincristine-induced painful neuropathy in rats: evidence of behavioral and biochemical examination. Nutritional Neuroscience.;20(1):60-70, 2017
10. **Komirishetty P, Areti A, Yerra VG, Ruby PK, Sharma SS, Gogoi R, et al.** PARP inhibition attenuates neuroinflammation and oxidative stress in chronic constriction injury induced peripheral neuropathy. Life sciences.;150:50-60, 2016
11. **Kim ST, Chung YH, Lee HS, Chung SJ, Lee JH, Sohn UD, et al.** Protective effects of phosphatidylcholine on oxaliplatin-induced neuropathy in rats. Life sciences. 130:81-7, 2015
12. **Heller, A. H., Eicher, E. M., Hallett, M. & Sidman, R. L**. Myotonia, a new inherited muscle disease in mice. *J. Neurosci.* **2**, 924–933. https:// doi. org/ 10. 1523/ JNEUR OSCI. 02- 07- 00924. 1982
13. **Zorniak, M., Mitrega, K., Bialka, S., Porc, M. & Krzeminski, T. F.** Comparison of thiopental, urethane, and pentobarbital in the study of experimental cardiology in rats in vivo. *J. Cardiovasc. Pharmacol.* **56**, 38–44. https:// doi. org/ 10. 1097/ FJC. 0b013 e3181dd502c, 2010
14. **Khodir, S. A., Abd-Elhafiz, H. I., Sweed, E. M., Gaafar, A. M., El-aziz, N. M. A., Salman, N. A. A., ... & Sheref, A.** ALuteolin ameliorates Indomethacin-Induced Gastric Ulcer in Rats via Activation of Nrf2/HO-1 Pathway. *Menoufia Medical Journal*, *37*(1), 17, 2024
15. **Kiernan J.A.** Histological and histochemical methods: Theory and practice, 5th edition in special stains and Hx& E: Folia Histochemica ET Cytobiologica; 54(1):58–59, 2015
16. **Paniagua N, Sánchez-Robles EM, Bagues A, Martín-Fontelles MI, Goicoechea C, Girón R.** Behavior and electrophysiology studies of the peripheral neuropathy inducedby individual and co-administration of paclitaxel and oxaliplatin in rat. Life Sciences.;277:119397, 2021
17. **Banach, M., Zygulska, A. L., &Krzemieniecki, K.** Oxaliplatin treatment and peripheral nerve damage in cancer patients: A Polish cohort study. *Journal of Cancer Research and Therapeutics*, *14*(5), 1010-1013, 2018
18. **Cavaletti G, Tredici G, Petrucciolo MG, Donde E, Tredici P, Marmiroli P, Minoia C, Ronchi A, Bayssas M, Etienne GG:** Effects of differential schedules of oxaliplatin treatment on the peripheral nervous system of the rat. Eur J Cancer, 37:2457-2463,2001

19. **Li, Z., Gao, Z., Sun, T., Zhang, S., Yang, S., Zheng, M., & Shen, H.** Meteorin-like/Metrnl, a novel secreted protein implicated in inflammation, immunology, and metabolism: A comprehensive review of preclinical and clinical studies. *Frontiers in immunology*, *14*, 1098570, 2023

20. **Ni W, Zheng X, Hu L, Kong C, Xu Q**. Preventing oxaliplatin-induced neuropathic pain: Using berberine to inhibit the activation of NF-κB and release of pro-inflammatory cytokines in dorsal root ganglions in rats. Experimental and Therapeutic Medicine;21(2):1-7, 2021

21. **Sommer C, Leinders M, Üçeyler N.** Inflammation in the pathophysiology of neuropathic pain. Pain. Mar;159(3):595-602. doi: 10.1097/j.pain.0000000000001122. PMID: 29447138, 2018

22. **Hu, J., He, A., Yue, X., Zhou, M., & Zhou, Y..** METRNL reduced inflammation in sepsis-induced renal injury via PPARδ-dependent pathways. *Food Science and Technology*, *42*, e61821, 2021

23. **Liu, N., Dong, J., Li, L., Zhou, D., & Liu, F**. The Function and Mechanism of Anti-Inflammatory Factor Metrnl Prevents the Progression of Inflammatory-Mediated Pathological Bone Osteolytic Diseases. *Journal of Inflammation Research*, 1607-1619, 2024.

24. **Semis, H. S., Kandemir, F. M., Caglayan, C., Kaynar, O., Genc, A., & Arıkan, S. M.** Protective effect of naringin against oxaliplatin-induced peripheral neuropathy in rats: A behavioral and molecular study. *Journal of Biochemical and Molecular Toxicology*, *36*(9), e23121, 2022.

25. **Kamboj, S.S., Sandhir, R.,** Protective effect of N-acetylcysteine supplementation on mitochondrial oxidative stress and mitochondrial enzymes in cerebral cortex of streptozotocin-treated diabetic rats. Mitochondrion 11(1), 214-222, 2011

26. **Kandemir, F.M., Yildirim, S., Caglayan, C., Kucukler, S., Eser, G.,.** Protective effects of zingerone on cisplatin-induced nephrotoxicity in female rats. Environ Sci Pollut Res Int 26(22), 22562-22574, 2019

27. **Delfan, M., Radkia, F., Juybari, R. A., Daneshyar, S., Willems, M. E., Saeidi, A., ... &Zouhal, H.** Unveiling the effects of interval resistance training and chlorella vulgaris supplementation on meteorin-like protein and oxidative stress in obese men. *Current developments in nutrition*, 104428, 2024

28. **Lee JO, Byun WS, Kang MJ, Han JA, Moon J, Shin MJ, et al.** The myokine meteorin-like (metrnl) improves glucose tolerance in both skeletal muscle cells and mice by targeting AMPKα2. Febs j.;287(10):2087-104, 2020

29. **Bito T, Okumura E, Fujishima M, Watanabe F.** Potential of Chlorella as a dietary supplement to promote human health. Nutrients.;12(9):2524, 2020

30. **Ta, L.E., Espeset, L., Podratz, J., Windebank, A.J., 2006**. Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology 27(6), 992-1002, 2020

31. **Cui, N., Li, S., Zhao, X., Zhang, T., Zhang, C., Yu, L., ... & Xie, K.** Expression of Bcl-2, Bax and Caspase-3 in nerve tissues of rats chronically exposed to 2, 5-hexanedione. *Neurochemical research*, *32*, 1566-1572, 2007

32. **Celik, H., Kucukler, S., Ozdemir, S., Comakli, S., Gur, C., Kandemir, F. M., &Yardim, A**. Lycopene protects against central and peripheral neuropathy by inhibiting oxaliplatin-induced ATF-6 pathway, apoptosis, inflammation and oxidative stress in brains and sciatic tissues of rats. *Neurotoxicology*, *80*, 29-40, 2020

33. **Li, J., Hong, Y., Zhong, Y., Yang, S., Pei, L., Huang, Z., ... & Wang, T.** Meteorin-like (METRNL) attenuates hypertensive induced cardiac hypertrophy by inhibiting autophagy via activating BRCA2. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1870*(4), 167113, 2024

34. **Hu, C., Zhang, X., Song, P., Yuan, Y. P., Kong, C. Y., Wu, H. M., ... & Tang, Q. Z.** Meteorin-like protein attenuates doxorubicin-induced cardiotoxicity via activating cAMP/PKA/SIRT1 pathway. *Redox Biology*, *37*, 101747, 2020

35. **Arafa, M.H., Atteia, H.H.** Protective Role of Epigallocatechin Gallate in a Rat Model of Cisplatin-Induced Cerebral Inflammation and Oxidative Damage: Impact of Modulating NF-kappaB and Nrf2. Neurotox Res 37(2), 380-396, 2020

36. **Xu, D., Lian, D., Wu, J., Liu, Y., Zhu, M., Sun, J., He, D., Li, L.,** Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis. J Neuroinflammation 14(1), 156, 2017

37. **Jorgensen JR, Fransson A, Fjord-Larsen L, Thompson LH, Houchins JP, Andrade N, *et al*.** Cometin is a novel neurotrophic factor that promotes neurite outgrowth and neuroblast migration *in vitro* and supports survival of spiral ganglion neurons *in vivo*. Exp Neurol; 233: 172–81, 2012

38. **Hong C, Wang Z, Zheng SL, Hu WJ, Wang SN, Zhao Y, et al.** Metrnlregulatescognitive dysfunction and hippocampal BDNF levels in d-galactose-induced aging mice. Acta Pharmacol Sin. doi: 10.1038/s41401-022-01009-y, 2022

39. **Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J.** Role of

Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci. 2016 Sep;73(17):3221-47. doi: 10.1007/s00018-016-2223-0. PMID: 27100828; PMCID: PMC4967105, 2016

40. **Nitti M, Piras S, Marinari UM, Moretta L, Pronzato MA, Furfaro AL.** HO-1 Induction in Cancer Progression: A Matter of Cell Adaptation. *Antioxidants*; 6(2):29. https://doi.org/10.3390/antiox6020029, 2017
41. **Campolo, M., Lanza, M., Paterniti, I., Filippone, A., Ardizzone, A., Casili, G., ... & Esposito, E.** PEA-OXA mitigates oxaliplatin-induced painful neuropathy through NF-κB/Nrf-2 Axis. *International Journal of Molecular Sciences*, *22*(8), 3927, 2021
42. **Feather, C. E., Lees, J. G., Makker, P. G. S., Goldstein, D., Kwok, J. B., Moalem-Taylor, G., & Polly, P.** Oxaliplatin induces muscle loss and muscle-specific molecular changes in Mice. *Muscle & nerve*, *57*(4), 650–658. https://doi.org/10.1002/mus.25966, 2018
43. **Sorensen, J. C., Petersen, A. C., Timpani, C. A., Campelj, D. G., Cook, J., Trewin, A. J., Stojanovska, V., Stewart, M., Hayes, A., & Rybalka, E.** BGP-15 Protects against Oxaliplatin-Induced Skeletal Myopathy and Mitochondrial Reactive Oxygen Species Production in Mice. *Frontiers in pharmacology*, *8*, 137. https://doi.org/10.3389/fphar.2017.00137, 2017
44. **Kamisli S, Ciftci O, Kaya K, Cetin A, Kamisli O, Ozcan C.** Hesperidin protects brain and sciatic nerve tissues against cisplatin-induced oxidative, histological and electromyographical side effects in rats. *Toxicology and Industrial Health*;31(9):841-851, 2015
45. **Zheng, S. L., Li, Z. Y., Song, J., Liu, J. M., & Miao, C. Y. Metrnl:** a secreted protein with new emerging functions. *Acta pharmacologicaSinica*, *37*(5), 571–579. https://doi.org/10.1038/aps.2016.9, 2016