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Epigenetic impact and ameliorative potential role of quercetin or rosemary extract on metalaxy or manganese chloride-induced toxicity via mitigation of microRNA, DNA methylation and regulation of MAPK phosphorylation in rats

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ABSTRACT

Metalaxyl fungicide play an important role in hepatotoxicity and liver damage. High levels of Manganese (Mn) exposure may cause irreversible brain disease. The potential protective role and epigenetic mechanism of Quercetin or rosemary extract in metalaxyl or manganese chloride ($MnCl_2$) toxicity were evaluated. Fifty-sex rats were split into two experiments. Experiment A: Metalaxyl hepetotoxicity 1/10 LD_{50} (130 mg/kg b.wt) and Experiment B: Manganese chloride neurotoxicity 1/25 LD_{50} (59.36 mg/kg b.wt). The experiment (A) divided into 4 groups: G1 (control group) given distilled water, G2 (Metalaxyl) received (130 mg/kg b.wt) three times a week for six weeks, G3 (Metalaxyl + Quercetin) given quercetin (50 mg/kg b.wt/day) and metalaxyl. G4 (Metalaxyl + Rosemary extract) given rosemary extract (200 mg/kg b.wt/day) and Metalaxyl. The experiment (B) split also into four equal groups similar to the design in experiment (A) but $MnCl_2$ (59.36 mg/kg b.wt) was given five times a week for six consecutive weeks. The results of metalaxyl exposed rats displayed up-regulation of liver MAPK1, miRNA-684 and DNA hypermethylation but, down-regulation of miRNA-7, up-regulation of miRNA-153 were detected in brain of $MnCl_2$ intoxicated rats compared to control. Quercetin or rosmary extract co-treatment with metalaxy significantly down-regulated liver MAPK1 and miRNA-684 with DNA hypomethylation with up-regulation of miRNA-7 and down-regulation of miRNA-153 in brain of $MnCl_2$ exposed rats. In conclusion, quercetin or rosmary extract displayed hepatoprotective and neuroprotective role against metalaxyl or manganese toxicity via mitigation of epigenetic markers MicroRNA, DNA Methylation and regulation of MAPK phosphorylation in liver and brain of rats.

Introduction

Metalaxyl is one of the harmful pesticides that are widely used, and statistics from around the world show that the usage of these chemicals for pest management is increasing. Generally, pesticides are applied directly to control pests that are present in both indoor and outdoor environments (Chhipa, 2017). Overuse of pesticides results in insufficient delivery systems and waste buildup, which alters the genetic structure of the pest species and eventually leads to insecticide resistance. Most pesticides are lost owing to pesticide drift, which puts the ecosystem at risk (Ayoub *et al*., 2017). Appealing substitutes are nanopesticides, which are used as "smart delivery systems" to release insecticides on schedule and under controlled conditions for a selected duration. By doing this, the likelihood of environmental pollution and the associated risks would decrease. Since nanoparticles may be non-specifically dangerous to both targeted and non-targeted organisms, acute toxicity testing should be performed (Srivastava *et al*., 2018).

Manganese (Mn) is the world's 4th most frequently utilized heavy metal and it resides in the II oxidation state, with Mn2+ and Mn3+ being the most common forms in biological systems (Kulshreshtha *et al*., 2021). It functions as an enzyme cofactor or activator for a variety of metabolic reactions and is a crucial trace element essential to the proper development of the human body. Even though Mn is necessary at trace levels, too much exposure to it can cause toxic accumulation in human brain tissue, causing extrapyramidal symptoms called manganism comparable to idiopathic Parkinson's disease (PD) (Bahar *et al*., 2017). Manganism is related to neurochemical changes, such as, excitotoxicity, impaired protein aggregation, mitochondrial dysfunction, oxidative stress, iron-homeostasis, and changed homeostasis involves the same transporters for other divalent metals as that of Mn++ (Huang *et al*., 2021). These alters have been contributed to dopaminergic degradation of neurons (Rai *et al*., 2021).

The term "epigenetics" refers to genetic alters that are heritable but do not include changes to the underlying DNA sequence. Moreover, epigenetics considers the impacts of environmental factors on genetic interactions throughout embryonic development (Irshad and Husain, 2021). The basic epigenetic regulatory covalent modifications involve the interference with DNA methylation/de-methylation, chromatin remodeling between others, and histone modifications, all of which impact gene expression and silencing, and in turn effect cellular processes as apoptosis, proliferation, and other malignant characteristics (Jakopovic *et al*., 2013). DNA methylation has been suggested as a crucial epigenetic mechanism for silencing genes linked with apoptosis, cell cycle, DNA repair, inflammation, and stress response. Generally, there are five types of DNA methyltransferases in humans accountable for establishing, and maintaining DNA methylation (DNAM). Involving DNA methyltransferase 1 (DNMT1), DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMTs activate the transfer of the methyl group at the fifth position of cytosine from S-adenosyl methionine into the dinucleotide CpG to form 5mC, which are called CpG islands (Paluszczak *et al*., 2010). Non-coding RNAs (ncRNAs) make up more than 75% of the mammalian genome and are essential for the regulation of numerous physiological and pathological processes. It also involves microRNAs (miRNAs), which are endogenous, small, single-stranded RNAs with a length of 21–25 nucleotides, and long non-coding RNAs (lncRNAs) that are nearly about 200 nt in length. Moreover, their regulatory role in various cellular processes as proliferation, inflammation, differentiation, immune response, and drug resistance is well recorded (Bayraktar and Van Roosbroeck, 2018).

Furthermore, Mitogen-activated protein kinase (MAPK) is the influential kinase in a highly specific three-layered kinase cascade with critical roles in deferent biological cellular processes, involving cell cycle progression, differentiation, and survival. Deregulation of this pathway is linked to numerous pathophysiological conditions. Recent studies have

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significantly increased our knowledge of the MAPK-ERK pathway signaling, permitting the development of diverse possible therapeutic strategies targeting the pathway. This special issue covers recent advances in the molecular mechanisms and functions of MAPK-ERK signaling in various biological contexts (Park, 2023).

In recent years, accumulating evidence has underscored the health-promoting impacts of numerous dietary phytochemicals. These impacts, mediated during diverse epigenetic mechanisms, hold significant promise for the treatment and prevention of many diseases (Koh *et al*., 2020). Specifically, dietary polyphenols, which are among the most studied plant metabolites are well regarded for their potential health advantages, which makes them excellent choices for the creation of chemoprevention efforts. Though polyphenols are a very broad and varied class of phytochemicals, flavonoids make up about 60% of all naturally occurring polyphenols (Ignat *et al*., 2011). Many bioactive substances have been found to be essential in the prevention, delaying, and reversal of disorders (Wang *et al*., 2019). Quercetin (QCT) (3,30,40,5,7-pentahydroxyflavone), a vital naturally occurring dietary polyphenol present in tea, red onions, berries, apples, red wine and citrus fruits (Zhang *et al*., 2015), has been shown to have potent anti-oxidant and anti-inflammatory properties, which may help prevent a variety of diseases, involving as cancer, diabetes, obesity, and neurological disorders (Lu *et al*., 2006). Because of its biological and pharmacological actions, which include antioxidant, anti-inflammatory, anti-carcinogenic, and antiviral qualities, quercetin is one of the most promising chemoprotective medicines against the harmful effects of environmental contaminants in experimental animals (Murakami *et al*., 2008). QCT plays a critical role in changing neurodegenerative diseases progression via its protective influence against oxidative stress (Dong *et al*., 2014).

Quercetin is a neuroprotectant that can cross the blood-brain barrier and lower brain cell apoptosis after focal cerebral ischemia (Paula *et al*., 2019). Currently, quercetin's neuroprotective properties are attributed to its capacity to inhibit apoptosis (Yao *et al*., 2012), oxidative stress (Ishisaka *et al*., 2011), and neuro-inflammation (Rinwa and Kumar 2013) by activating the Akt signaling (Lei *et al*., 2015). Numerous studies have identified the possible intracellular targets of quercetin. The mechanisms involved are primarily attributed to the control of oncogene expression, the induction of apoptosis in malignant cells, and the modification of tumor angiogenesis via the Wnt/β-catenin, MAPK/ERK1/2, PI3K/Akt/mTOR pathways (Reyes-Farias and Carrasco-Pozo, 2019). Additionally, quercetin's potential beneficial health impacts may be related to the epigenetic targets as it can be applied as an epigenetic regulator in variety disease models (Tan *et al*., 2009).

Rosemary (*Rosmarinus Officinalis*) is a medicinal herb belonging to the Labiatae family whose active extracts are contain mostly of 1,8-cineole, carnosic acid, carnosol, and rosmarinic acid, β- and α-pinene, flavonoids, camphor, steroids, diterpenes, triterpenes, and camphene (Bao *et al*., 2020). According to several studies, rosemary extract may have antioxidant, hepatoprotective, antithrombotic, diuretic, antidiabetic, antinociceptive, anticancer, and anti-inflammatory properties in both individuals and experimental animals (Moore *et al*., 2016). Because it includes phenols and flavonoids, rosemary leaf extract has beneficial antioxidant effects (Shin *et al*., 2016). This study was designed to investigate the potential role of epigenetic mechanism in metalaxyl induced hepatotoxicity and manganese chloride induced neurotoxicity and their intracellular pathways alterations in the liver and brain of rats. Moreover, the possible protective effect of quercetin and rosemary extract on liver and brain malfunction in rats were also explored.

Materials and methods

Experimental Animals

Fifty-six male albino rats, weighing between 100 and 150 g at 4-5 weeks of age, were gotten from Laboratory Animals Research Center, Faculty of Veterinary, Benha University. Animals were kept in separated stainless steel cages and kept up for 12 hours, light/dark cycle, (22 ± 3°C) and (48 ± 8) % relative humidity. Water and food were given ad- libitum. Rats were kept for two weeks before the experiment's beginning to adapt to the place. The Experimental protocol was done with the guidelines for Institutional Animals Care as well as Use Committee and approved by Research Ethics Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM 07-02-23).

Chemicals and natural agents

Metalaxyl: Metalaxyl, N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)-DL-alanine methyl ester, technical grade of 98% was obtained from Zhejiang Heben Pesticide and Chemicals Co., Ltd. China. Metalaxyl was dissolved in 1.72 mL of dimethyl sulfoxide (DMSO) then; 22.4 mL of propylene glycol was added. Freshly dissolved metalaxyl compound was administrated at the dose of 130 mg/kg b.wt (1/10 of LD_{50}) three times in the week for 6 weeks orally (Sakr and Lamfon, 2005).

Manganese chloride 99%: Manganese chloride tetrahydrate ($MnCl_2$. $4H_2O$) was produced by Advent Chembio Pvt. Ltd, Navi Mumbai, India. Rats were given manganese orally for a period of six weeks at a dose of 1/25 LD_{50} (59.36 mg/kg b.wt) diluted in distilled water and given five times in the week (Vez´er *et al*., 2005).

Quercetin: {2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran- 4-one dihydrate, 3,3',4',5,7-Pentahydroxyflavone dehydrate). Quercetin was gotten from Aktin Chemicals, Inc. company (Nature connecting health); Chengdu; China.

Preparation of Quercetin stock solution:400 mg of quercetin was added to 2 ml of DMSO. 1 ml of quercetin DMSO stock solution was added to 2 ml Tween 80 and diluted to obtain 14 ml using normal saline. Each rat received 1 ml of this solution orally (Indap *et al*., 2006). For six weeks, rats were administered a daily dosage of 50 mg/kg b.wt of quercetin.

Rosemary: Rosemary was provided from Al-Harraz Co for Agriculture Seeds, Herbs and Medicinal plants, Cairo, Egypt.

Preparation of Rosemary leaves extract: To avoid decaying of chemical components of the dried rosemary leaves about 200 g were grained into a fine powder. Flowing that, the plant powder was added to ethanol [ethanol/water (70:30)] in a stoppered container, the mixture was kept standing for a minimum of three days at room temperature. After that, the mixture was clarified to make a liquid extract, which was then concentrated at 50°C and low pressure by a rotary evaporator. This procedure was repeated three times at least. Finally, the extract was weighted and stored at -20°C until it was used. 0.5 ml from rosemary extract was given orally/daily for each rat (Abdel-Gawad *et al*., 2021).

Experimental Design

Rats were separated into two main experimental groups as follows: Experimental group A: Metalaxyl-induced liver toxicity at a dose of 1/10 LD_{50} (130 mg/kg b.wt). Experiment group B: Manganese chloride-induced neurotoxicity at a dose of 1/25 LD_{50} (59.36 mg/kg b.wt).

Experiment group A

Twenty-eight rats were separated into four equal major groups, each with seven rats, as follow: G1 (Control): rats did not receive any treatment. G2 (Metalaxyl): Rats administrated oral dosages of metalaxyl 1/10 LD_{50} (130 mg/kg b.wt) three times a week for six weeks. G3 (Metalaxyl + Quercetin): Rats were administered quercetin orally (50 mg/kg b.wt/day) for a continuous six weeks and administrated metalaxyl (130 mg/kg b.wt) three times a week. G4 (Metalaxyl + Rosemary extract): Rats were administered rosemary extract (200 mg/kg b.wt/day) orally (Al-Attar and Shawush, 2014) for a continuous six weeks, and administrated metalaxyl (130 mg/Kg b.wt)

three times a week.

Experiment group B

Twenty-eight rats were divided into four groups, each group consisted of seven rats. G1: (Control): Rats were supplemented with distilled water. G2: (Manganese chloride): Rats were given manganese chloride at a dose of 1/25 LD_{50} (59.36 mg/kg body weight) orally, five days a week for a duration of 6 weeks. G3: (Manganese chloride + Quercetin): Rats were administered quercetin orally at a dose of 50 mg/kg b.wt per day, for six weeks, along with five weekly doses of manganese chloride at a dose of 59.36 mg/kg b.wt. G4: (Manganese chloride + Rosemary extract): Rats were given Rosemary extract daily for six consecutive weeks at a dose of 200 mg/kg b.wt (Al-Attar and Shawush, 2014), and five weekly doses of Manganese chloride at a dose of 59.36 mg/kg b.wt orally.
N.B: Throughout the experimental period, the dose was adjusted every week according to any alteration in the body weight to preserve similar dose per kg body weight of rat over the entire period of experiment for each group.

Liver and brain tissue specimens

At the end of the six-week experiment, rats were euthanized in according to Animal Ethics Committees and their abdomens were opened then the livers tissue specimens of (Experiment A) were collected. Also, the skull was opened and the brain specimens of (Experiment B) were immediately collected. Both liver and brain were rinsed with sterile saline solution to eliminate any blood cells and clot, then moved to Eppendorf tubes, saved in liquid nitrogen rapidly, and kept at -80°C until RNA extraction for gene expression analysis using RT-PCR.

Molecular analysis

Determination of MAPK1 gene expression

The mRNA expression levels of MAPK1 in the livers of rats were estimated using real-time qPCR . Using a complete RNA purification kit and the manufacturer's instructions, pure RNA was removed from liver tissues (Thermo Scientific, Fermentas, #K0731). Every cDNA sample was reverse transcribed via used the Revert Aid TM First Strand CDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA). Next, using gene-specific primers and the manufacturer's protocol (Thermo Scientific, USA, # K0221), RT-PCR with SYBR Green was utilized to evaluate gene expression. The target genes expression normalizes with glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) using 2-ΔΔCt technique (Livak and Schmittgen, 2001). The sequences of forward (/5 ---- /3) and reverse primers (/5 ---- /3) for MAPK1 and GAPDH genes used in RT-qPCR is AGGGCGATGTGACGTTT/CTGGCAGGGTGAAGTTGG and CAACTCCCTCAAGATTGTCAGCAA / GGCATGGACTGTGGTCATGA, respectively.

Determination of miRNA-684, miRNA-7 and miRNA-153 gene expression

For evaluation of miRNA-684 expression in the liver, miRNA-7 and miRNA-153 in the brain, Real- time PCR and SYBR green were used with U6 acting as inside management. According to the manufacturer's instructions using (USA, Thermo Scientific, # K0221), the extracted cDNA was amplified, the universal reverse primers integrated with the Quanti-Mir RT kit and a miRNA-specific forward primers sequence (/5 ----- /3) for primers used for miRNA-684, miRNA-7, miRNA-153 and U6 were AACTGAACTTCCCTTTTGA, TGGAAGACTAGTGATTTTGTTGT, TTGCATAGTCACAAAAGTGATC and TGACACGCAAATTCGTGAAGCGTTC, respectively.

Determination of global DNA methylation

Assessment of global DNA methylation in liver tissue was directed using the Colorimetric Base Catalog # P-1030 method. The level of global DNA methylation was valued using the MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit from EpiGentek in Farmingdale, NY, USA, following the protocol by (Li *et al*., 2018).

Statistical analysis

All the data were expressed by the means ± SE. One-way analysis of variance (ANOVA) was used to assess the statistical significance by using SPSS, 18.0 software 2011, and the Duncan's multiple range test (DMRT) was used to produce individual comparisons. The statistical significance was well-known as $p \leq 0.05$.

Results

Protective Impact of Quercetin or Rosemary extract on the relative expression of MAPK1 and mRNA-684 genes and global DNA methylation in the liver of Metalaxyl –intoxicated rats

The results of the qPCR analysis presented in Figure 1 showed the livers of rats intoxicated with metalaxyl, and displayed a notable up-regulation of the liver MAPK1 and miRNA-684 gene expression level when compared to the control normal group. On the other hand, treatment with quercetin or rosemary extract significantly down-regulate liver MAPK1 and miRNA-684 gene expressions compared to the metalaxyl exposed. Moreover, a significant global DNA hypermethylation was observed in rats exposed to metalaxyl. In contrast, treatment with quercetin or rosemary extract in metalaxyl-intoxicated rats resulted in significant global DNA hypomethylation when compared to metalaxyl untreated group (Figure 2).

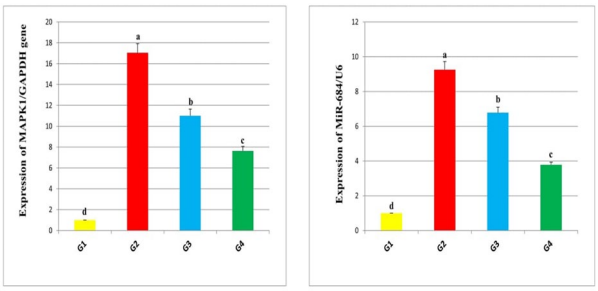

Figure 1. Graphical presentation of RT-qPCR analysis of liver MAPK1 and mRNA-684 gene expressions in metalaxyl-intoxicated rats after quercetin or rosemary extract treatment.

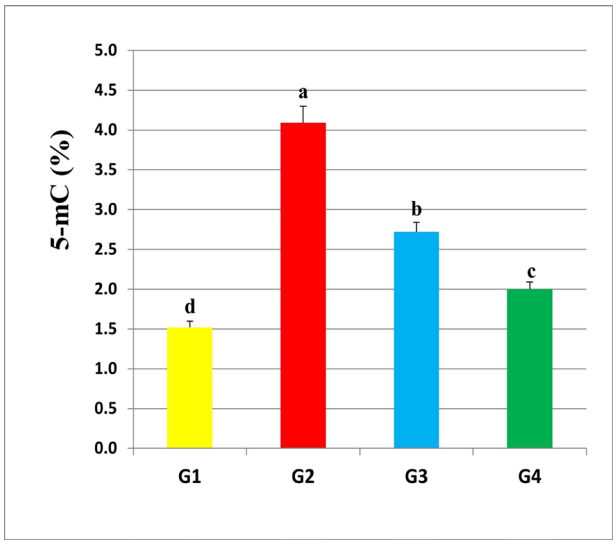

Figure 2. The impact of quercetin or Rosemary extract on Global DNA methylation level in the liver of Metalaxyl intoxicated rats

Protective impact of Quercetin or Rosemary extract on the relative expression of mRNA-7 and mRNA-153 genes in the brain of Manganese chloride-intoxicated rats

The results of the qPCR analysis presented in Figure 3 showed a significant down-regulation in the expression of miRNA-7 and up-regulation of miRNA-153 genes in the brains of rats exposed to manganese chloride compared to the control group. However, the gene expression levels of brain miRNA-7 were significantly up-regulated and miRNA-153 was significantly down-regulated in rats treated with quercetin or rosemary extract after manganese intoxication when compared to untreated rats.

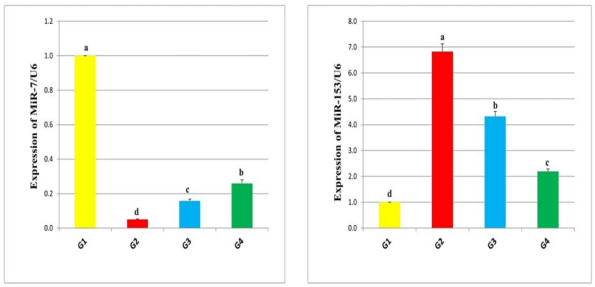

Figure 3. Graphical presentation of RT-qPCR analysis of brain mRNA-7 and mRNA-153 gene expressions in Manganese chloride intoxicated rats after quercetin or rosemary extract treatment.

Discussion

It has been demonstrated that the generation of oxidative stress is one of the main processes driving pesticide-induced damage. Although a variety of drugs are used to treat pesticide intoxication, their usage has been limited because of side effects. The effort to identify naturally occurring antioxidants and verify their capacity for reducing oxidative stress resulting from pesticide exposure continues to garner attention. As important members of the polyphenol family, flavonoids exhibit strong antioxidant properties (Zeng *et al*., 2021).

The obtained results indicated that rats exposed to metalaxyl displayed a notable up-regulation of the liver MAPK1 and miRNA-684 gene expression and global DNA hypermethylation when compared to the control group. Key signal transducing enzymes that control a variety of biological processes are called MAPKs. Several MAPKs activate specific effector kinases as MAPK-activated protein kinases (MAPKAPKs) and are inactivated by MAPK phosphatases. C Jun NH2-terminal kinase (JNK/SAPK), extracellular signal-regulated kinases 1 and 2 (ERK1/2), and p38 kinase are three conventional MAPKs controlling important cellular processes involving gene expression, translation, mRNA stability, cell proliferation and differentiation, survival and apoptosis (Cargnello and Roux, 2011). Similarly, Fong *et al.* (2017) reported that, an up-regulated ERK/MAPK signaling pathway may cause apoptosis via activating apoptotic factors, involving cas8. Moreover, ERK/MAPK signalling pathway regulates the cell cycle of hepatocytes; thus, up-regulated ERK/MAPK phosphorylation might prevent the proliferation of hepatocytes and disturb the development of the liver (Guégan *et al*., 2012). Also, Lee *et al.* (2024) displayed that, hexaconazole causes developmental toxicities via inflammation, apoptosis, and changes of MAPK and Akt signaling cascades. These findings suggested that alterations in the MAPK and Akt signaling pathways may trigger other toxic mechanisms and prevent organ formation. Therefore, controlling the MAPK and Akt signaling pathways might be a breakthrough in ameliorating the toxicities triggered by hexaconazole fungicide in zebrafish. Furthermore, exposure to Chlorothalonil (CTL) induced several impacts on organisms and in particular its reproductive toxicity has been attracted public concern. Additionally, the CTL-induced oxidative stress activated MAPK pathway and injured the maturation of oocytes (Wang *et al*., 2024).

Nonetheless, treatment with quercetin or rosemary extract significantly down-regulate liver MAPK1 and miRNA-684 gene expressions with significant global DNA hypomethylation compared to the metalaxyl untreated rats. The potential intracellular targets of quercetin have been described in different studies, and mechanisms are linked with the regulation of the oncogene expression, induction of malignant cells apoptosis, and modulation of tumor angiogenesis through Wnt/β-catenin, PI3K/Akt/mTOR, MAPK/ERK1/2 pathways (Reyes-Farias and Carrasco-Pozo, 2019). The protective impacts of quercetin on carbon tetrachloride (CCl4)-induced liver fibrosis in rats and to clarify its anti-hepatofibrotic mechanisms were recorded by Wang *et al*. (2017), it reduced the expression of p38 MAPK by suppressing its phosphorylation. The Inhibitory impacts of quercetin on the progression of liver fibrosis through the regulation of p38 MAPK, NF-κB/IκBα, and Bcl-2/Bax signaling. An essential member of the MAPK super family is p38 MAPK, which plays a role in the response of hepatic stellate cells (HSCs) to liver injury and inflammation (Ito *et al*., 2006). Suppression of p38 MAPK phosphorylation can prevent HSCs activation, resulting in inhibition of liver fibrosis development (Gu *et al*., 2016). Many studies have shown that QCT can prevent the p38 MAPK signaling pathway. Consequently, Appling quercetin to target p38 MAPK or NF-κB signaling pathway may be a treatment strategy to prevent fibrosis. Thus, it was speculated that therapeutic influence of quercetin on liver fibrosis could be mediated through suppressing NF-κB and p38 MAPK signaling pathways, preventing inflammatory response, and inhibiting liver cell apoptosis (Wang *et al*., 2017). As we expected, this research showed QCT can prevent the expression of p38 MAPK and p-p38 MAPK, indicating that quercetin could inhibit the progression of liver fibrosis through regulating the p38 MAPK signaling pathway.

Furthermore, studies on cardiac fibroblasts have shown that QCT reduces the phosphorylation of ERK, p38 and JNK induced by free radicals, but there have only a few studies which investigated the relationship between quercetin and MAPK pathway in mammary epithelial cells (Min *et al*., 2019). In this study, H2O2 activated the p38 MAPK, ERK and JNK molecules by phosphorylation, and quercetin restored the activation of these molecules, revealing that MAPK pathway might play a role in the protective impacts of quercetin in mammary epithelial cells. Similarly, quercetin reduced Nuclear factor erythroid 2-related factor 2 (Nrf2) p-NRF2/NRF2 ratio in cells treated with H2O2, proving that Nrf2 pathway may also likely to be included in quercetin's protective role to oxidative stress. These results are consistent with the reports that quercetin is a Nrf2-interacting nutrient in improving Alzheimer's disease, lung injury, and insulin resistance (Bousquet *et al*., 2020; Martín-Acosta and Xiao, 2021; Zarrin *et al*., 2021).

Carnosic acid is a phenolic diterperne compound found in abundance in sage and rosemary, which are both widely used in traditional medicine. Research over the past decade suggests that carnosic acid has multiple bioactive properties involving anti-inflammatory, antioxidant, and anticancer activities among others. Rosemary extract rich in carnosic acid (CA) and rosmarinic acid (RA) was recorded to cure bleomycin-(BLM)-induced pulmonary fibrosis. Bahri *et al.* (2016) reported that, using carnosic acid modulation of MAPK p38, PI3K/Akt pathways and activation of both caspase-dependent and caspase-independent signaling. CA and carnosol (CAR) are two major diterpenes of the rosemary plant. They possess a phenolic structural moiety and are endowed with the power to remove cellular ROS either through direct scavenging reaction or indirectly through up-regulation of antioxidant defences (Habtemariam *et al*., 2023). Rosemary extract and its main polyphenolic components RA, CA and CO has been shown to prevent the PI3K-Akt, MAPK, and NF-κB pathways and their linked pro-inflammatory mediators in vitro in different other immunological cells, as well as in vivo in animal models (Yousef, 2018). The suppression of the MAPK and PI3K-Akt pathways is of a vital importance to prevention the pro-inflammatory process that occurs in allergen activated mast cells. Both signaling pathways are included in activation of downstream transcription factors, cytokines, and chemokines which work to drive inflammation, tissue damage, and allergy at the site

of insult (Kim, 2014).

Also, Wang *et al*. (2012) reported that, EGFR/MAPK signaling plays an important role in the inhibitory impacts of CA on hepatocyte lipid accumulation. Likewise, Kang *et al*. (2021) investigated that, the signalling pathway linked with the anti-inflammatory activity of CA (2.5–20 μM) in Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Moreover, MAPKs involving ERK, JNK, and p38, along with NF-κB, and FoxO signalling pathways, were inhibited, as evidenced by the inactivation of IKKβ/IκB-α/NF-κB, MAPKs and FoxO1/3. Furthermore, the down-regulation of IKK activity by CAR (5 μM) was further shown to be linked with inhibition of LPS-induced phosphorylation as well as degradation of IκBα. Correspondingly, Seyedemadi *et al*. (2016) observed that R. officinalis L. hydroethanolic inhibited the blood-brain barrier rupture, as well as infarction, the cerebral edema, and neurological problems, in a murine model with middle cerebral artery occlusion. This contributed to the ability of R. officinalis L. to inhibit the MAPK phosphorylation, which allows the blockade of NF-kB activation. This blocking will reduce the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). CA and CAR display anti-inflammatory impacts and enhance cell survival through regulation of the MAPK pathways (Wang *et al*., 2020; Westenberger *et al*., 2021).

The existing findings showed a significant downregulation of miRNA-7 and upregulation of miRNA-153 expression levels in brain of $MnCl_2$ exposed rats. MicroRNAs (miRNAs) are 17–24 base small non-coding, single stranded RNAs that control gene expression post-transcriptionally by binding to the 3′-untranslated region (UTR) of mRNAs, thereby repressing the translation process (Gartel and Kandel, 2008). It has recently been determined that dysregulation of miRNA expression raises the risk of neurodegeneration (Johnson *et al*., 2012). Altered expression of microRNAs, including miR-29a/b-1, miR-19, miR-101 and miR-130 has been linked to neurodegenerative disorders (Hébert *et al*., 2008). Furthermore, it has been shown that miR-7 and mir-433 control the expression of SNCA in both normal and PD brain, as well as in animal models of PD, such as, the worm C. elegans (Asikainen *et al*., 2010; Junn *et al*., 2009).

Simultaneously, down-regulation of mi-R-7 and mi-R433 was shown in a prior work following Mn exposure. The exact mechanism causing this down-regulation is unknown. However, Mn has been shown to induce the death of neurons resulting from oxidative stress may induce a dysregulated miRNA expression (Tarale *et al*., 2018). The oxidative stress (OS) resulting from Mn exposure has a critical to change epigenetic regulation of α-synuclein via hypomethylation of SNCA gene promoter or through downregulation of miR-7/miR-153 (Tarale *et al*., 2016). Furthermore, OS-induced α-synuclein overexpression by several pathways, such as ERK/MAPK activation, induces epigenetic alters in MiR-7/MiR-153 expression and hypomethylation of the CpG island of the SNCA gene at intron-1 (Junn *et al*., 2009). The known pathways connected to Mn-induced neurotoxicity are (1) misfolding of proteins (such α-synuclein and amyloid), (2) oxidative stress, mitochondrial dysfunction, and (3) neuroinflammation (Yan *et al*., 2019). The expression of miR-222 and miR-21 is increased in the peripheral blood of workers exposed to particulate matter containing Mn, As, Fe, Ni, Pb, Cr, and Cd (Bollati *et al*., 2010). In Mn-exposed neuronal cell lines, downregulation of miR-7 and miR-433 was closely linked with the upregulation of SNCA, thereby causing neuronal apoptosis (Tarale *et al*., 2018). Wang *et al*. (2023) indicated upregulated miRNA-nov-1 can enhance Mn-induced apoptosis in N27 cells, which was regulated via Dhrs3 activated mTOR signaling pathway.

As a widely expressed miRNA molecule in the mammalian brain, microRNA-7 (miR-7) has a well-established involvement in the development of several diseases. Crucially, a growing body of research has demonstrated that miR-7 is implicated in several brain disease and developmental processes. Expressively, sensory or neurosecretory neurons are the primary sites of expression for miR-7, which is encoded by three genes on separate chromosomes. Furthermore, three processes—gene transcription, primary and precursor sequence development, and mature sequence formation—control the expression of miR-7. From a physiological perspective, miR-7 primarily controls the development of the pituitary gland, optic nerve system, and cerebral cortex. From a pathological standpoint, miR-7 has the ability to regulate numerous genes, which allows it to manipulate the course of different brain diseases such as neurodegenerative diseases, neuroinflammatory diseases, and mental disorders (Zhao *et al*., 2020). Also, significantly, various recent studies have found that miR-7 is highly enriched in brain tissue and is closely linked to physiological and pathological process of brain (Smigielski *et al*., 2020), indicating that it plays an important novel role in brain tissue development and disease occurrence, hence, it is may be a new promising therapeutic target for brain diseases. Similarly, Tarale *et al*. (2018) shown that, Mn-exposure causes deregulation of certain miRNAs. Expression of miR-7 and miR-433 was down-regulated following Mn exposure. These miRNAs could be useful therapeutic targets since they may be essential for preventing the neurotoxic process caused by manganese.

Moreover, miR-7 can block NF-κB signaling pathways to shield neurons (primary neurons in mice, human neural progenitor cells ReNcell VM cells, and dopaminergic SH SY5Y cells) from the cytotoxicity caused by MTPT, indicating that elevated levels or miR-7 activity may represent a novel therapeutic strategy for PD (Choi *et al*., 2014). Furthermore, by blocking the mTOR signaling pathway, miR-7 can raise the expression of the anti-apoptotic protein molecule BCL2, preventing the damage to neuronal cells caused by MTPT in PD model (Fragkouli and Doxakis, 2014). The protective effect of miR-7 and miR-133b against experimental PD caused by MPP + and atrazine raises the possibility of using miRNAs therapeutically (Long *et al*., 2012).

MiRNA-7 and miRNA-153 have the ability to block MPP+-induced neuronal death by notably controlling α-Syn translation. Elevating the ROS levels in the mitochondria could highlight how miR-7 and miR-153 regulate α-Syn (Je *et al*., 2017). Clinically, Wu *et al*. revealed high plasma levels of miR-153 and miR-223 in PD patients (Wu *et al*., 2022). The upstream mechanisms causing down-regulation of miR-7 and miR-433 are unknown. However, Mn exposure is known to induce neuronal cell apoptosis through OS mechanisms (Fernandes *et al*., 2017). Our present study suggests a role formiR-7 in Mn-induced neurodegeneration by causing SNCA overexpression. Our results raise the possibility of using miR-7and miR-433 as therapeutic targets to prevent or treat manganese induced neurotoxicity.

Meanwhile, the significant upregulation of miRNA-153 expression levels in brain of $MnCl_2$ exposed rats. Similarly, a previous investigation recorded that hypoxia-stimulated endoplasmic reticulum stress enhances miR-153 transcription via activating IRE1α and its downstream transcription factor X-box binding protein 1 (XBP1). XBP1 directly binds to the promoter of the miR-153 host gene PTPRN (Liang *et al*., 2018). Also, Ectopic expression of miR-153-3p caused inflammation by increasing the produce of TNF-α, IL-1β, and IL-6 and reduced neural stem cell differentiation via regulating GPR55 expression (Dong *et al*., 2023). Increased expression of miR-153 disrupted synapsin 1 in the hippocampus and impaired glutamatergic vesicle transport thus enhancing chronic cerebral hypoperfusion in rats (Zhang *et al*., 2020). Meanwhile, other studies have demonstrated beneficial impact of increasing miR-153. In this respect, Xu *et al*. (2019) found that an increased expression of miR-153 can protects the neurons from cellular death via the upregulation of PRX5. Similarly, miR-153–3p reduces LPS-induced neuroinflammation and subsequently cell death by preventing the NF-κB signaling pathway (Choi *et al*., 2022).

In the current study Quercetin or Rosmary extract co-treatment with $MnCl_2$ induce significant upregulation of brain miRNA-7 and downregulation of miRNA-153 gene expression. MiR-7 is an mRNA of α -synuclein, which contributes to the etiology of PD. Our method identified a natural product QCT as a molecule able to up-regulate cellular miR-7 levels and down-regulate the expression of α –synuclein (Zhu *et al*., 2021). MiR-7 also targets other genes implicated in PD, involving RelA, Nlrp3 or Sir2 (Zhao and Wang, 2019). Zhu *et al*. (2021) indicated that, QCT interrupts HuR binding with both pri-miR-7–1 and α -Syn mRNA, promoting miR-

7 biogenesis, suppressing α -Syn translation and reducing α -Syn transcripts levels. All of these together contribute to the strong repression on α - Syn expression by QCT (Zhu *et al*., 2021).

The influence of rosemary on neurotoxicity has been reported. Balawi *et al*. (2010) observed that treatment with rosemary extracts ameliorated acrylamide-enhanced neurotoxicity in all brain areas of albino rats (involving the brain striatum, stem, cerebellum, hippocampus, cerebral cortex, and hypothalamus). Also, de Oliveira *et al*. (2015) illustrated the role of carnosic acid in avoiding neurotoxicity caused by methylglyoxal in SHSY5Y neuroblastoma cells through increasing anti-oxidative actions and activating PI3K/Akt/Nrf2 pathway. Rosemary extract acts as a prophylaxis for different neurodegenerative diseases induced by oxidative stress or apoptosis, for example, Parkinson's disease (Park *et al*., 2010). Furthermore, Azhar *et al*. (2023) reported that, rosmarinic acid shows neuroprotective impacts on neurons in neurodegenerative diseases, restores loss of function in neurons by decreasing free radical-mediated OS, neuroprotective role against OS in N2A cells and decreases Amyloid-β-induced neurotoxicity (Azhar *et al*., 2023). Moreover, Sepand *et al*., (2013) displayed that rosmarinic acid shows neuroprotective influences by decreasing OS and inhibiting brain cell deaths in vitro against different neurological diseases and neurotoxic molecules. Moreover, Luft *et al*. (2019) revealed that RA exhibited antioxidant activity, reduced ROS production, superoxide dismutase activity, DNA damage, and neuroprotective impacts. In present study rosemary extract significantly increase mRNA-7 in brain tissue, mRNA-7 plays a crucial regulatory role in brain diseases. MiR-7 and miR-153 are known to suppress α-synuclein, and are therefore protective by reducing its associated neurotoxicity and neuroinflammation (Fragkouli and Doxakis, 2014). Choi *et al.* (2014) proven that miR-7 protects neurons by targeting and suppressing the expression of RelA and alleviating the inhibition of NF-kB. Additionally, miR-7 can inhibit 1-methyl-4-phenylpyridinium [MPP(+)]-caused cell death by down-regulating NF-kB p65 (RelA), elevating glucose transporter 3 (Glut3) expression, and encouraging glycolysis (Chaudhuri *et al*., 2015). Furthermore, miR-7 protected against MPP(+)-caused apoptosis in neuronal cells by directly targeting Krüppel-like factor 4 (KLF4) (Kong *et al*., 2016). Also it prevented MPP(+)-caused neuronal cell apoptosis by directly targeting B-cell lymphoma-2-associated X protein (Bax) and sirtuin2(Sirt2) (Li *et al*., 2016). Furthermore, miR-7 could prevent EGFR/STAT3 pathway and TLR4 expression to block brain microglia activation, cytokine production and decrease the secondary damage of brain tissue in a Cerebral Hemorrhage model (Zhang *et al*., 2018). Moreover, miR-7 could govern the pathology of brain tissue inflammation through controlling the inflammatory reaction of neuronal cells in brain tissue inflammation model (Yue *et al*., 2020). Therefore, rosemary extract has beneficial effects on neurodegenerative diseases through enhancing antioxidant activities and enhancing miR-7.

Conclusion

The present study provides evidence for the hepatoprotective and neuroprotective properties of quercetin and rosemary extract against metalaxyl or manganese chloride toxicity in rats. It also improves liver injury epigenetic markers, which may be related to a decrease in DNA hypermethylation, miR-684, and the prevention of liver MAPK phosphorylation. The present work discusses the possible correlation between reduced levels of miR-7 and miR-153 caused by manganese exposure and altered miRNA expression in neurodegenerative disorders. These miRNAs could be useful therapeutic targets since they may be essential for preventing the neurotoxic process caused by manganese.

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Conflict of interest

The authors declare that they have no conflict of interest.

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