

Taxifolin ameliorates IL-17A-enhanced hippocampal inflammation and oxidative stress in arsenic-exposed HT-22 cells

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Abstract

Arsenic (As) promotes neuronal damage in the hippocampus which is associated with cognitive function. Taxifolin, a flavonol with strong anti-oxidative and anti-inflammatory properties, is known to have beneficial properties in neurodegeneration, but its effect on As-induced hippocampal damage is unknown. Here, we explored whether taxifolin has therapeutic potential in ameliorating as causing toxicity in mouse hippocampal HT-22 cells, hypothesizing that inhibiting oxido-inflammation stress may reverse As-associated damage through several pathways. Findings indicate that pretreatment with taxifolin reversed the reduction of sodium arsenite (NaAsO₂) induced cell death. In addition, taxifolin ameliorated NaAsO₂-related oxidative stress as measured by the formation of ROS, MDA level and GSH content. Moreover, we revealed the taxifolin treatment resulted in a reduction of IL-17A level and subsequent diminished activation of TNF- α , IL-1 β , and IL-6 concentrations in HT-22 cells treated with NaAsO₂. These findings imply when considered collectively, HT-22 cells are shielded by taxifolin from NaAsO₂-evoked oxidative cell death and neuroinflammation. Thus, our report indicates that has a protective role in hippocampal cell culture systems.

Keywords: Arsenic; Hippocampus; Inflammation; Neurotoxicity; Oxidative damage

1. Introduction

Arsenic (As) is a crucial environmental toxicant to which millions of human beings are exposed worldwide owing to the depletion of drinking water with high levels of as [1]. As is able to cross the blood-brain barrier, and its elevated concentration within the brain deregulates neurocellular functions [2]. Epidemiological findings have declared that exposure to As could influence intellectual and cognitive skills in children [3, 4]. Pandey et al. showed that rats exposed to As exhibited deficits in learning and memory, as well as neuronal loss activated by apoptosis [2].

The hippocampus provides memory functions, and hippocampal neuronal degeneration forms a considerable reason for cognitive dysfunction [5]. It is demonstrated that As-induced preliminary morphological changes within the hippocampus connected with learning-memory dysfunction [2, 6].

Oxidative stress prompted by excessive buildup of reactive oxygen species (ROS) participates in the occurrence and development of neurological diseases and injuries [7]. Experimental research on rodents illustrated that exposure of As led to elevating lipid peroxidation (LPO) as well as reducing the activities of antioxidants in the hippocampus, which are present to fight ROS [8, 9]. The overgenerating of ROS in As poisoning makes an antioxidant disequilibrium which stimulates a reduction in the potential of the mitochondrial membrane, which causes the production of pro-inflammatory cytokines [10, 11].

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Interleukin 17A (IL-17A), a crucial member of IL-17 family, has received considerable attention for its pro-inflammatory role in neurodegenerative disease [11]. IL-17A promotes secretion of pro-inflammatory cytokines, like IL-1 β and IL-6, which aggravates the inflammatory response including cerebral ischemia-reperfusion injury and Alzheimer's disease [11, 12]. According to recent research, IL-17A leads to impaired neurocognitive functions by activating downstream inflammatory pathways which may amplify inflammation via the secretion of IL-1 β and IL-6 cytokines [13]. In addition, Yang et al reported that anti-IL-17A treatment alleviates neuroinflammation by reducing TNF- α , IL-1 β , and IL-6 proinflammatory cytokines and oxidative damage via strengthening antioxidant defense mechanism in the hippocampus [14]. However, there has been no report in the literature about the role of IL-17A in hippocampal neurodegeneration induced by As exposure. Therefore, more research is needed to better assess the potential risks associated with As.

Chelation therapy with antioxidant supplementation is recommended to treat As-related poisoning, which is an important health problem [15]. Thus, it is imperative that novel chemicals be thoroughly examined for their potential to treat As neurotoxicity and other associated dysfunctions.

Taxifolin is a flavonoid compound, that originally existed in many plants like French maritime pine bark and milk thistle and is also used in commercial preparations [16]. Taxifolin has a variety of pharmacological actions, the most essential among which are antioxidant and anti-inflammatory features [17]. Iwasa et al. revealed that taxifolin suppresses IL-1 β generation by reducing the intracellular ROS levels in inflammatory responses to high-glucose-stimulated mouse microglial cells [18]. It is also reported taxifolin treatment with cerebral amyloid angiopathy group mice resulted in a reduction in LPO and oxidative stress-responsive genes in the hippocampus tissue [19]. All this information indicates that taxifolin may possess the ability to treat As-related hippocampal neurodegeneration. In addition, no information was found in the literature investigating the protective effect of taxifolin against As-induced hippocampal damage. It is in this context that we investigate the current report on the protective role of taxifolin in As-related neurotoxicity in hippocampal mouse HT-22 cells.

2. Material and methods

2.1. Cell culture

HT-22 cells, a mouse hippocampal neural cell line, were kindly donated by Asst. Prof. Caner Gunaydin (Samsun University, Samsun, Turkey). In DMEM (Grand Island, NY, USA) containing 10% FBS, 1% penicillin-streptomycin, and 2 mM l-glutamine (Grand Island, NY, USA), cells were cultured at 37 °C in a humidified environment with 95% air and 5% CO₂. Experiments were carried out with cells at 80 % confluence. For cell viability and ELISA test, HT-22 cells were seeded in 96 and 24 wells.

2.2. Drug treatments and cell viability

Cell viability of HT-22 cells was determined with MTT (Sigma-Aldrich, St. Louis, MO) test. HT-22 cells were seeded in 96 wells at a density of 10,000 cells/well and cultured in the incubator overnight. The following day, fresh medium was used to replace the culture medium with different concentrations of taxifolin (Sigma-Aldrich, St. Louis, MO) (final concentration 20, 40, and 80 μ g/mL) for 1 h prior to exposure to NaAsO₂. After that, the cells were exposed to NaAsO₂ (Sigma-Aldrich, St. Louis, MO) (final concentration 10 μ M) for 24 h [2, 20]. Then the medium was removed, and cells were applied with MTT (1 mg/ml) solution for 4 hours (5% CO₂; 37 °C). DMSO (100 μ l) was used for dissolving purple formazan crystals. Cell viability (%) was computed with optical density (OD) defined at 570 nm with a spectrophotometer (Multiskan GO, Thermo Scientific, USA) [21].

2.3. Biochemical analysis

The oxidative markers, ROS, malondialdehyde (MDA), and glutathione (GSH), concentrations were measured with commercial kits in conformity with kits protocol (LSBio, Texas, USA; Elabscience, Texas, USA; respectively). The levels of tumor necrosis factor-alpha (TNF- α), IL-17A, IL-1 β , and IL-6 (Elabscience, Texas, USA) were quantified with commercially available ELISA kits pursuant to the manufacturer's guidelines. By comparing their respective absorbance values to the standard curve, these parameter concentrations in the samples were determined. Bovine serum albumin was used as a standard for the Bradford assay, which measures protein concentrations.

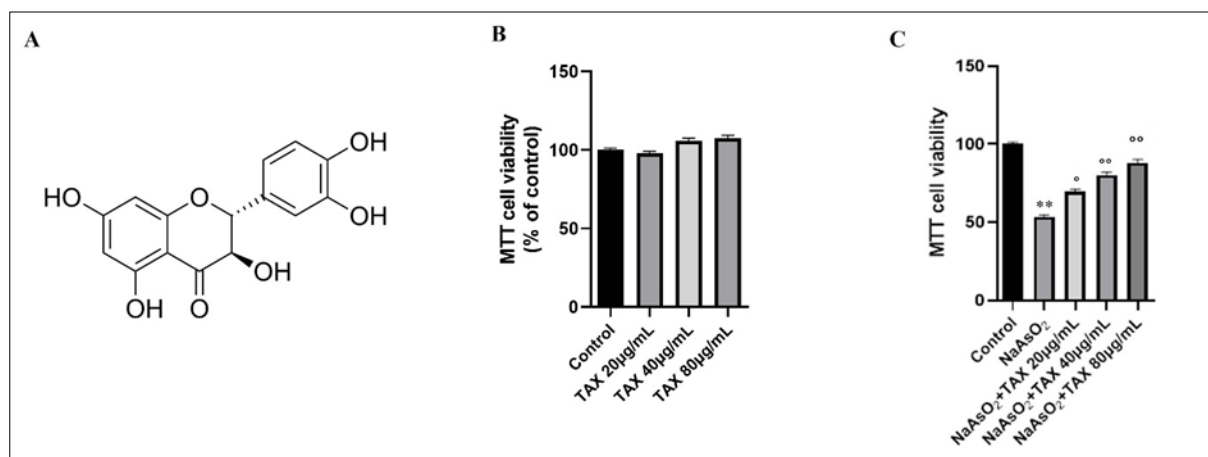
2.4. Statistical analysis

The findings obtained in this report were statistically evaluated using IBM SPSS Statistics (Version 22.0, IBM Co., Chicago, IL, USA) software. One-way ANOVA was used to statistically analyze the data, and then the Tukey post-hoc test was performed. A P-value less than 0.05 was accepted as statistically meaningful.

3. Results and discussion

3.1. Taxifolin Inhibits NaAsO₂-induced HT-22 Hippocampal Cell Death

Analysis of cell viability with an MTT test was carried out to evaluate whether the taxifolin displays considerable protective performance towards NaAsO₂ causing neuronal damage. Firstly, we investigated the effects of taxifolin on HT-22 cells viability. After cells were exposed to taxifolin alone at concentrations of 20, 40, and 80 µg/mL did not lead to any apparent cytotoxicity evaluated by MTT assay. Cell viability in HT-22 cells at 20, 40, and 80 µg/mL taxifolin doses were found 97.8%, 105.8%, and 107.3%, respectively (Figure 1B). And then, after being applied with 10 µM NaAsO₂ for 24h, the cell viability was remarkably reduced to 53.5%, in comparison to control cells ($P < .0001$) (Figure 1C). Wang et al. reported that 10 µM NaAsO₂ significantly reduced viability in HT-22 cells, supporting our findings [20]. However, the pre-treatment with taxifolin for 1 h ameliorated the cytotoxic effects. 20, 40 and 80 µg/mL taxifolin significantly decreased NaAsO₂-prompted cytotoxic effects, and the cell viability increased to 69.4% ($P < .01$), 80.3% ($P < .0001$), and 88.2% ($P < .0001$), respectively. Applying of taxifolin especially at the dose of 80 µg/mL increased cell viability the most. The elevated cell viability obtained in taxifolin-treated NaAsO₂ groups revealed the potential protective feature of taxifolin against NaAsO₂-induced cell damage in HT-22 cells. These findings underline the specificity of taxifolin keeping hippocampal neuronal cells from NaAsO₂ leading damage.



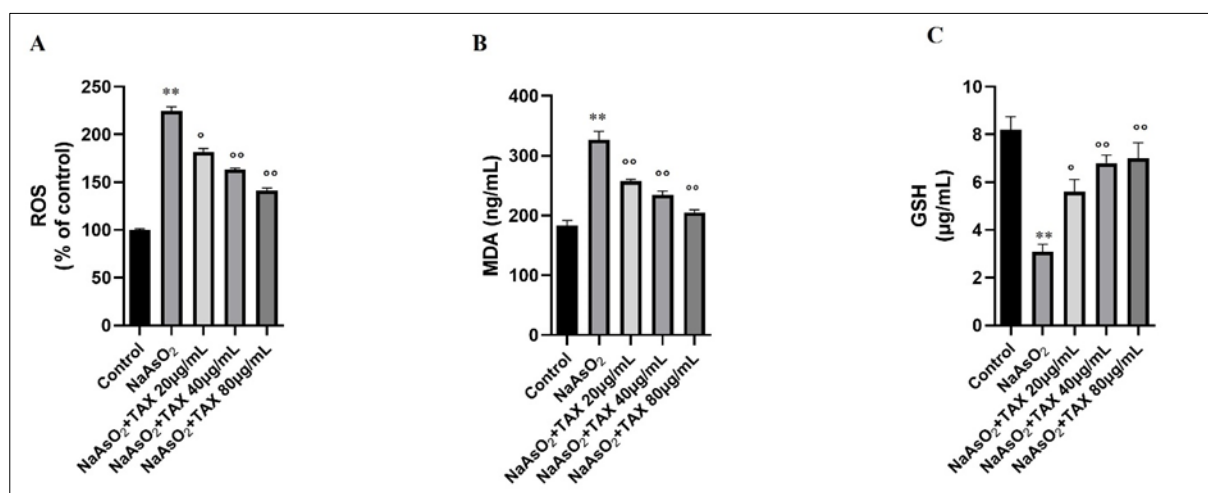
(A) The structure of taxifolin (Molecular formula: C₁₅H₁₂O₇; Molecular weight: 304.25); (B) HT-22 cells were incubated with different concentrations of taxifolin for 24 h; (C) HT-22 cells were incubated with or without taxifolin (20, 40 and 80 µg/mL) for 1h, followed by incubation with 10 µM NaAsO₂ for another 24 h. Cell viability was evaluated with MTT test. Data were given as mean ± S.D. of three experiments in triplicate (n = 9). ** $P < .0001$ vs. control; ° $P < .01$ and °° $P < .0001$ vs. 10 µM NaAsO₂

Figure 1 Taxifolin inhibits NaAsO₂-induced HT-22 cell death

3.2. Taxifolin Alleviates Oxidative Stress Promoted by NaAsO₂

Oxidative stress has been implicated as a major culprit in As toxicity, and therapeutic approaches based on improving the clearance of highly radical species in cells hold great promise for attenuating As- evoked toxicity [9]. An elevation of ROS concentration in the brain hippocampus is one of the earliest reactions to As toxicity that can harm cellular antioxidant defenses [22]. As leads to LPO via ROS related to oxidative injury to polyunsaturated fatty acids (PUFA) [8, 9]. MDA, a by-product of LPO, is generated under conditions of oxidative injury. It represents oxidative damage of the plasma membrane and the happenings substances of thiobarbituric acid reactive are connected to oxidant stress and LPO [23]. An important antioxidant enzyme, called GSH, has been indicated to detoxify LPO and hydrogen peroxide by the catalytic action of glutathione peroxidase and thus protects cells from ROS damage [24]. Therefore, we tested the hippocampal cells on the levels of intracellular ROS generation, production of MDA, and GSH content after cells were treated with NaAsO₂. As shown in Figure 2A-B, after being applied with 10 µM NaAsO₂, intracellular ROS and MDA levels were dramatically elevated, while GSH content (Figure 2C) was importantly decreased in comparison to those of control hippocampal cells ($P < .0001$) suggesting that NaAsO₂ could induce oxidative damage. These outcomes are in agreement with the findings stated by Keshavarz-Bahaghighat et al. [22]. And then, we investigated whether taxifolin could

alleviate As-induced oxidative stress. When HT-22 cells were pre-treated with taxifolin at all concentrations (20, 40 and 80 $\mu\text{g}/\text{mL}$) for 1h, followed by exposure to 10 μM NaAsO_2 for 24 h, intracellular ROS level and MDA level were markedly reduced as compared with the NaAsO_2 group (20 $\mu\text{g}/\text{mL}$ taxifolin for ROS and MDA: $P < .01$; 40 and 80 $\mu\text{g}/\text{mL}$ taxifolin for ROS and MDA: $P < .0001$). Furthermore, taxifolin also mitigated NaAsO_2 -induced decline in GSH (20 $\mu\text{g}/\text{mL}$ taxifolin: $P < .01$; 40 and 80 $\mu\text{g}/\text{mL}$ taxifolin: $P < .0001$), indicative of a reduction of oxidative stress. This could be the consequence of taxifolin's capacity to ravage As-evoked ROS by electrons being transferred, metals chelation, and stimulating antioxidant enzymes. There are no studies showing the effect of taxifolin on As-induced neurotoxicity. However, a previous study revealed that taxifolin treatment of rats with hepatic encephalopathy makes inhibition of ROS generation and MDA levels as well as an elevation in the content of GSH replenishment and improved antioxidant defense in hippocampus cells, which was in support of our work [17]. Taxifolin through inhibition of production of free radicals and restoration of oxidized membrane phospholipids displayed profitable effects against oxidative injury [25]. Furthermore, the outcomes in Figure 2 were compatible with the cell viability assay (Figure 1C) attesting to the specific protective impact of taxifolin upon NaAsO_2 evoked damage in HT-22.



(A) intracellular ROS level, (B) MDA level, (C) GSH level. Data were given as mean \pm S.D. of three experiments in triplicate (n = 9). ** $P < .0001$ vs. control; * $P < .01$ and $^{\circ\circ}$ $P < .0001$ vs. 10 μM NaAsO_2

Figure 2 Effect of taxifolin on biomarkers of NaAsO_2 -induced oxidative stress in HT-22 cells

3.3. Taxifolin Suppress IL-17A and Proinflammatory Cytokines Elevation in NaAsO_2 -Treated HT-22 Cells

Accretion of ROS as a consequence of As-promoted oxidative stress can interfere with inflammation pathways through protein kinase C activation as evidenced by elevated levels of pro-inflammatory cytokines [15]. IL-17A is a pivotal inflammatory mediator exacerbating chronic tissue inflammation, triggering multiple signaling molecules like $\text{TNF-}\alpha$, IL-1 β , and IL-6 [13, 14]. To explore the specific role of taxifolin on hippocampal inflammation in NaAsO_2 -treated cells, we evaluated IL-17A and its related inflammatory cytokines including $\text{TNF-}\alpha$, IL-1 β and IL-6. The exposure of HT-22 cells for 24 h to NaAsO_2 brought an increase in IL-17A levels ($P < .0001$; Figure 3A). To our knowledge, the literature contains no reports indicating the role of IL-17A in hippocampal toxicity caused by As. However, it is reported that mice with diabetic encephalopathy display higher IL-17A levels in the hippocampus which induces lower cognitive ability, degeneration of hippocampal neurons, and higher expression of inflammatory factors including $\text{TNF-}\alpha$, IL-1 β compared to control [26]. As shown in Figure 3, after being treated with 10 μM NaAsO_2 , $\text{TNF-}\alpha$ ($P < .0001$; Figure 3B), IL-1 β ($P < .0001$; Figure 3C), and IL-6 ($P < .0001$; Figure 3D) levels were significantly increased along with the elevation of IL-17A demonstrating that NaAsO_2 could promote inflammation. As evidenced in the current report, the level of IL-17A besides activated other cytokines was associated with the As-related inflammatory response in the hippocampus. As was demonstrated to augment the levels of inflammatory cytokines inclusive of $\text{TNF-}\alpha$, IL-1 β and IL-6 in rats's hippocampus [17]. Pre-treatment with taxifolin at all concentrations for 1h followed by exposure to 10 μM NaAsO_2 for 24 h significantly declined the levels of $\text{TNF-}\alpha$, IL-1 β , and IL-6 in comparison to those of only NaAsO_2 exposure HT-22 cells ($P < .05$). Taxifolin showed the most effective anti-inflammatory effects at a concentration of 80 $\mu\text{g}/\text{mL}$. According to a hypothesis, in addition to the antioxidant action, taxifolin can modulate inflammation responses in the hippocampus [17, 27]. Hu et al. reported that taxifolin can reduce some inflammatory parameters in spinal cord injury model in rats, reflecting its anti-inflammatory properties, which was in support of our report [28]. In a recent study done by Iwasa et al., taxifolin treatment suppressed microglia activation, generation of inflammatory mediators, and elevation of intracellular ROS levels [18]. All these data suggest that taxifolin reduces neuroinflammation through its anti-inflammatory properties.

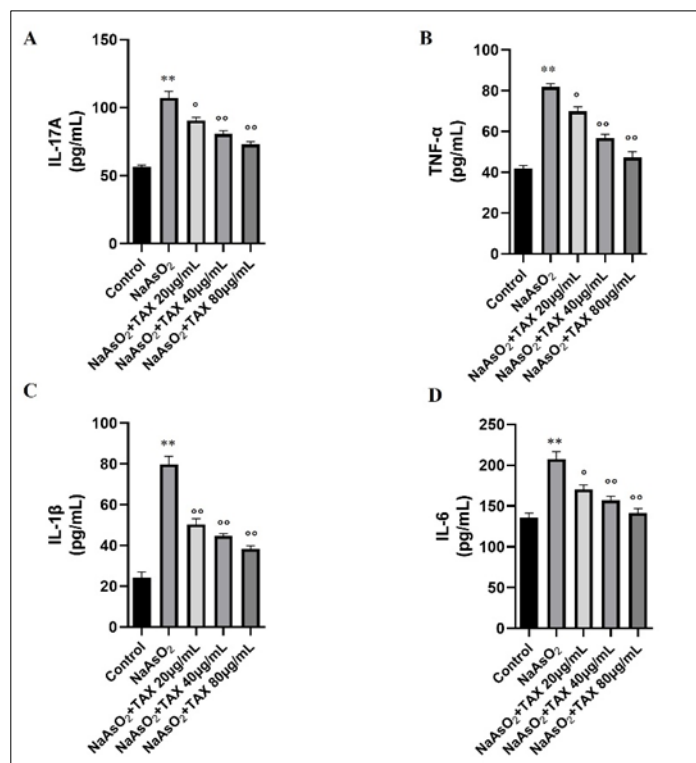


Figure 3 Effect of taxifolin on biomarkers of NaAsO₂-induced inflammation in HT-22 cells. (A) IL-17A level, (B) TNF-α level, (C) IL-1β level, and (D) IL-6 level. Data were given as mean ± S.D of of three experiments in triplicate (n = 9). ** P < .0001 vs. control; ° P < .01 and °° P < .0001 vs. 10 µM NaAsO₂

4. Conclusion

The current report reveals, for the first time, taxifolin alleviated hippocampal neurodegeneration in the As-induced HT-22 cells in vitro model. The neuroprotective effect of taxifolin is attributable to alterations in oxidative stress markers and proinflammatory cytokine levels, as well as its potential effects on IL-17A. In view of the above findings, we propose that taxifolin can be a powerful candidate for diet supplementation in company with chelation therapy in As-induced neurotoxicities as it demonstrates all propensities to regulate hippocampal-related oxidative damage and inflammation associated with it. These outcomes may shed light on further studies.

Compliance with ethical standards

Disclosure of conflict of interest

The authors state that there are no interests at odds with one another.

Statement of ethical approval

Since the HT-22 cell line was utilized in our investigation, ethical committee permission is not necessary.

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