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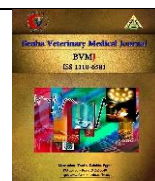
*Original Paper*

# Ameliorative effect of novel nanocompound against oxidative stress-induced brain toxicity in mice

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## ARTICLE INFO

## ABSTRACT

This study investigates the antioxidant properties of pH-responsive chitosan-based poly (acrylamide/acrylic acid) hesperidin (Cs/P(AAc/AAm)/HSP) Nanogel in AlCl3+NaF-intoxicated mice brain tissues as a pre-treatment and post-treatment agent. Fifty adult male Albino mice were used in our study and were divided equally into 5 groups of (I) normal control, (II) Cs/P(AAc/AAm)/HSP, (III) AlCl3+NaF, (IV) pre-treatment (prophylaxis), (V) post-treatment (therapeutic). We used the colorimetric technique to detect the concentrations of MDA and GSH as well as the activity of SOD and GPx. AlCl3+NaF induced severe neurotoxicity and oxidative stress in mice brain tissue, manifested by the marked changes in the studied parameters. Either pre- or post-Cs/P(AAc/AAm)/HSP oral administration ameliorated these changes, as demonstrated by the significant reduction in MDA concentration, and increased GSH levels. Additionally, Cs/P(AAc/AAm)/HSP restored the activity of SOD and GPx enzymes, indicating mitigation of the induced oxidative stress. Our findings from this study clearly showed the antioxidant potential of Cs/P(AAc/AAm)/HSP either as a prophylactic or therapeutic agent against AlCl3+NaF-induced brain damage.

## 1. INTRODUCTION

Fluoride (F) and aluminum (Al) are widely distributed metals found on earth. The unavoidable environmental exposure to both often results in adverse health effects. F, the essential element, is physiologically active while it penetrates tissues and cells because of its high biological activity and is present in microorganisms, plants, animals, as well as human beings (Štepec and Ponikvar-Svet, 2019). Fluoride is a monovalent anion and occurs usually in the form of sodium or aluminum-fluoride, NaF is the common fluoride salt. The previous studies suggest that less than 1 ppm of F is useful in dental caries prevention, but if the F concentration increases more than 1.5 ppm, it causes fluorosis (Srivastava and Flora, 2020). Al does not exist in a pure form, but commonly it is present in a combined form with fluorine, oxygen, silicon, phosphate, sulfate, hydroxide, or other elements. However, it occurs in trace amounts in biological material; it is not a useful element and is mostly considered to be harmful to human health (Faroon et al., 2012). Al enters our body via daily food intake, cooking utensils, drinking water, pharmaceuticals e.g., Al-containing antacids and vaccines, and through industrial exposures (Finke et al., 2015).

Both Al and F toxicity cause a marked imbalance in the redox system and as a consequence generate the reactive oxygen species (ROS) that have unpaired electrons; these are extremely reactive and hence, can cause severe damage to the cell nucleic acid, proteins, lipids, and carbohydrates (Kinawy, 2019a; Fernandes et al., 2021). They are known among the common neuro-toxicants that induce CNS disturbances and subsequently behavioral instabilities (Kinawy, 2019b). The injurious effects of F and Al on CNS are also attributed to their potential to easily cross the blood-brain barrier BBB and then they are absorbed and accumulated in the different brain regions. This will cause several degrees of cerebral damage and the occurrence of neurological diseases later (Saunders et al., 2012; Dórea, 2015). Oxidative stress is an imbalance between ROS and the enzymatic and non-enzymatic antioxidants that protect our body. When the concentration of ROS is not well controlled by the internal defense system, it leads to damage to lipids, proteins, and DNA, which causes toxicity in the body. Antioxidants are likely to offer a protective effect in their induced tissue damage.

Citrus flavonoids are considered reasonably safer and can pass the BBB (Khan et al., 2020). Hesperidin (HSP) acts as an antioxidant and has free hydroxyl (-OH) groups that can release electrons into ROS. HSP has been studied for its beneficial properties such as anti-hypertensive, capillary protective, anticarcinogenic, antiallergic, gastroprotective, anti-fatty liver, neuroprotective, metal-chelating, antidiabetic, antiviral, antifungal activities (Küçükler et al., 2021; Kuzu et al., 2021). Some of the

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benefits of HSP use are its safety, non-accumulative nature, and limited side effects, even during pregnancy. HSP has poor water solubility and oral bioavailability (Cao et al., 2018). Our team designed chitosan-based nanogel for HSP delivery to overcome this problem.

We hypothesize that exposure to both F and Al could impact the brain negatively. Besides, we also propose that HSP might have a potential effect on the brain redox status in this model. Therefore, in our work, we studied the effects of Cs/P(AAc/AAm)/HSP Nanogel against F and Al-induced neurotoxicity in mice and in this regard, we focus on redox status as a possible mechanism underlying this effect.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Sodium fluoride (NaF) was purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA) provided Al-gomhoria Co. (Cairo) supplied aluminum chloride ($AlCl_3$). All reagents, including acrylamide (AAm), acrylic acid (AAc), and dimethyl sulfoxide (DMSO), were acquired from Sigma-Aldrich Co., Hesperidin (HSP) (95%) from Al-dawlya Co., and Chitosan (Cs) from Techno-Gen, Gisa, Egypt.

Preparation of chitosan-based poly (acrylamide/acrylic acid) hesperidin (Cs/P(AAc/AAm)/HSP) Nanogel by gamma radiation, and calculation of LD50 was according to (Deiab *et al.,* 2023). Cs/P(AAc/AAm)/HSP Nanogel was administered at a dose (20 mg/kg b.w.), by oral gavage.

### 2.2. Experimental design

The experimental protocol and all animal caring procedures were carried out according to the Animal Ethical Committees of Benha University Guidelines, with an ethical approval number (BUFVTM 12-11-22). 50 adult male Swiss albino mice with an average weight of 25-35 g were purchased from El Nile Pharmaceutical Co. During the experiment period, mice were allowed *ad libitum* access to water and food, and housed under the same conditions with a light-dark cycle of 12 h, temperature of 22 ± 2 °C, and humidity of 50 ± 15%. After the acclimatization period, Mice were classified into 5 equal groups of

i. Group I (Control group): mice did not receive any treatment.

ii.        Group II (Cs/P(AAc/AAm)/HSP group): mice received Cs/P(AAc/AAm)/HSP Nanogel (20 mg/kg b.w.) using oral gavage, for 14 days.

iii.        Group III ($AlCl_3$+NaF group): mice received orally $AlCl_3$ (200 mg/kg b.w.) + NaF (10 mg/kg b.w), in drinking water, daily for 30 days.

iv.        Group IV: mice administered orally with Cs/P(AAc/AAm)/HSP Nanogel (20 mg/kg b.w.) for 14 days, then, on the 15th day rats will be received  $AlCl_3$ + NaF as mentioned before in group III for 30 days.

v. Group V: mice received $AlCl_3$ + NaF as mentioned before in group III for 30 days, and then rats will be

administered with Cs/P(AAc/AAm)/HSP Nanogel as mentioned before in group II for 14 days.

The doses of NaF and $AlCl_3$ were based on the LD 50 of fluoride in male mice (54.4 mg-F/kg b.w.) and of $AlCl_3$ (400 mg-Al/kg b.w.) (Chinoy *et al.*, 2005).

After 45 days, the mice were starved overnight, anaesthetized with (24 mg/kg b.w. i.m. injection), and sacrificed by decapitation. Serum blood samples were taken. Brain tissues were extracted and utilised for biochemical examination.

### 2.3. Biochemical analysis

The activity of SOD and GPx, and the concentration of GSH and MDA were determined by the colorimetric method exactly as described in the commercial kits (Bio-diagnostic, Egypt).

### 2.4. Statistical analysis

We used the statistical program SPSS (Statistical Package for the Social Sciences) version 20.0 to analyze the data. For the multiple comparisons, we utilized one-way ANOVA followed by a post hoc test.

## 3. RESULTS

3.1. Characterization of Cs/P(AAc/AAm)/HSP Nanogel
Dynamic light scattering was used to validate the pH influence on the size and zeta potential of the produced Cs/p(AAc/AAm)/HSP solution, while a transmission electron microscope (TEM) was used to analyze the nanogel size and shape (Deiab *et al.,* 2023). 3.2. Effect of Cs/P(AAc/AAm)/HSP Nanogel on concentrations of MDA and GSH in all groups.
Table (1) illustrates the effects of Cs/P(AAc/AAm)/HSP Nanogel on oxidative stress status of the brain tissue were profound in the context of toxicity caused by $AlCl_3$+NaF. The $AlCl_3$+NaF intoxicated group showed a significant increase in MDA a long with a significant decrease in GSH concentrations  compared to control group, indicating heightened lipid peroxidation. Nanogel-treated groups either pre- or post-treatment showed a marked reduction in the lipid peroxidation product (MDA) and a significant elevation of GSH levels indicating the attenuation of the oxidative stress, as compared to $AlCl_3$+NaF intoxicated group.

Table (1): Effect of Cs/P(AAc/AAm)/HSP Nanogel on MDA and GSH concentrations in brain tissues of $AlCl_3$+NaF-intoxicated rats.

| Groups | Parameters | |
|---|---|---|
|  | MDA ( nM/ g tissue) | GSH (mg/ g tissue) |
| Control | 57.8 ±2.4 [d] | 19.0 ±0.7 [b] |
| Cs/p(AAc/AAm)/HSP | 55.9 ±2.4 [d] | 20.9±0.8 [a] |
| $AlCl_3$+NaF | 155.1±5.1 [a] | 4.4±0.1 [e] |
| Pretreatment (with HSP Nanogel) | 80.3 ±3.0 [c] | 13.9±0.6 [c] |
| Posttreatment (with HSP Nanogel) | 103.2± 3.6 [b] | 10.2±0.5 [d] |

Data were presented as mean ± standard error mean (SEM), n = 5. Means in the same column had different superscript letters are significantly different at *p ≤ 0.05.*

3.3. Effect of Cs/P(AAc/AAm)/HSP Nanogel on activities of SOD and GPx in all groups.
Fig. 1 indicates that SOD and GPx activities are significantly inhibited *(P< 0.05)* in the $AlCl_3$+NaF group, indicating a drop in antioxidant defence mechanisms when compared to the control group. However, whether

provided as a pre- or post-treatment, Cs/P (AAc/AAm) / HSP Nanogel efficiently normalised these oxidative indicators. When compared to the intoxicated group, pre- and post-HSP Nanogel treated groups showed substantial *(P< 0.05)* increases in SOD and GPx activities, indicating increased antioxidant capability. All these data highlight the Cs/P(AAc/AAm)/HSP Nanogel's significant antioxidant capability, as it prevented the oxidative stress caused by $AlCl_3$+NaF.
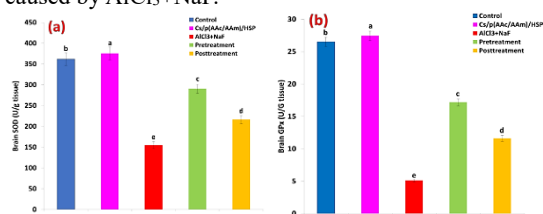


Fig. 1. Oxidative stress biomarkers (a) SOD and (b) GPx activity in all groups. Data were presented as mean ± standard error mean (SEM), n = 5. Means in the same column had different superscript letters are significantly different at *p ≤ 0.05*.

## 4. DISCUSSION

Our results showed that co-exposure to aluminum and fluoride, which are widely distributed metals, induced excessive oxidative stress in brain tissue, which can be observed by the significant increase in the MDA levels, with significant declines in GSH content as well as SOD and GPx activity in the $AlCl_3$+NaF- intoxicated group, when compared with the control group. Oxidative stress is a well stablished mode of action of both F and Al toxicities that has been reported in several in vitro and in vivo studies (Akinrinade et al., 2015). Numerous studies have investigated the influence of Al and F separately or as a combination on the redox status (Kinawy and Ezzat, 2013; Thangapandiyan and Miltonprabu, 2013; Nalagoni and Karnati, 2016). The pro-oxidant effects of F and Al that we recorded were previously demonstrated in the studies which showed that Al and F enhance CNS lipoperoxidation injury with the associated alterations in the enzymatic as well as the nonenzymatic redox miliu. Our results come in agree with Nalagoni and Karnati, (2016) who reported that $AlCl_3$+NaF co-administration for mice induces neuronal damage and severe oxidative stress marked by decreased SOD and CAT activity, along with elevated MDA production. Moreover, Kinawy, (2019) reported that exposure to Al or F either alone or in combination during the prenatal period and up to until the age of 70 days for rats caused significant decreases in GSH, vitamin C, and the GSH/GSSH ratio in the hippocampus, cerebral cortex, and hypothalamus of the offspring; along with triggered lipid peroxidation as well as increase in GSSH, and nitric oxide levels, with much more profound effect in the groups received aluminum and fluoride combined treatment. Kaur et al., (2009) suggested that aluminum enhances the neurotoxic hazards induced by fluoride, and they showed that concomitant exposure to both elements not only affect oxidative status, but also it resulted in profound alterations in neurotransmitters content.

The inhibited GPx activity is presumably attributed to the decrease in GSH concentration, GSH is a key cofactor for the action of this enzyme. The GSH and the GSH-GSSG ratio are essential markers of the redox status. The glutathione in its reduced form is a vital agent in reducing ROS, that is capable of detoxifying most harmful toxicants. The SOD enzyme decreased activity is a straight threat to the cellular integrity, as its deficit causes a serious imbalance in the cellular redox system resulting in oxidative stress, peroxidation, several degenerations, as well as mitotic cell death. Lipid peroxidation has a serious bearing on the CNS functions and structure, resulting in immense neurodegenerative disease (Kaur et al., 2009).

Earlier studies have revealed that fluorosis results in pathological damage to the CNS and cognitive dysfunction (Jaiswal et al., 2020). In this context, it was reported that chronic fluorosis caused GSH depletion and triggered the superoxide anion production in the brain of a rat model (Ma et al., 2014)

Similarly, co-exposure to F and Al has been shown to induce lipid peroxidation and decrease the levels of non-enzymatic antioxidant glutathione as well as the activity of the antioxidant enzyme liver (Chinoy et al., 2004; Sharma et al., 2010), blood (Wen et al., 2019), and reproductive organs (Patel and Shahani, 2020). Either fluoride (Shuhua et al., 2012; Xu et al., 2013) or aluminum (Wu et al., 2012; Fernandes et al., 2021) exposure enhanced ROS generation, which sounds principally essential in intermediating their effects. While aluminum is a non-redox metal it can modify the redox potential, affecting the natural redox process of the living system (Fernandes et al., 2021; Zhou et al., 2022). It was previously reported that Al directly restricts SOD activity, affects the activity of $Na^+/K^+$-ATPase, and triggers lipid peroxidation in the brain of rats (Silva et al., 2005; Kinawy and Ezzat, 2013). The chief production sources of reactive oxygen species in the central nervous system are the microglial cells as well as the astrocytes (Sheng et al., 2013). Former studies have demonstrated that high ROS levels cause several neurodegenerative pathologies. Hence, effective anti-oxidant agents may exert neuroprotective effects by decreasing ROS levels and inducing anti-inflammatory cascades (Tabner et al., 2001).

Hesperidin has a variety of intriguing protective effects in neurons and glial cells that are relevant to CNS diseases. In vivo and in vitro neurodegenerative models, HSP has been shown to protect neurons against cytotoxicity caused by oxidative stressors and neurotoxic chemicals (Roohbakhsh et al, 2014). In this study, improvement in the oxidative stress markers in brain tissues was observed in the Cs/P(AAc/AAm)/HSP Nanogel prophylactic as well as therapeutic groups. It was shown that HSP treatment significantly mitigated the increment in MDA concentration and the reduction in GSH level, compared to the intoxicated group. Also, It was determined that HSP exhibited its antioxidant effect by increasing the activity of antioxidant enzymes SOD, and GPx, compared with the control group. Our results are matched with Jaiswal et al., (2020), who recorded that HSP treatment alleviated F-induced redox imbalance by decreasing ROS, thiobarbituric acid reactive substances (TBARS) levels, along with elevating the GSH content in the brain, they suggested the involvement of peroxisome proliferator-activated receptor gamma (PPAR-γ) receptor in HSP neuroprotective effect against F-induced neurotoxicity. Previously, it was reported that HSP can pass the BBB,

decrease ROS level, inhibit lipid peroxidation in the cerebral cortex, and reduce oxidative stress by enhancing the activity of SOD, GPx, and catalase (CAT) (Hong and An, 2018). In a similar study, HSP reduced lipid peroxidation and improved the activities of CAT, SOD, GPx, and GSH levels in NaF-induced brain tissue (Yıldız et al., 2022). Previous studies showed that HSP prevented brain lipid peroxidation, and depletion of the reduced glutathione, and maintained their normality in AlCl₃-exposed mice (Jangra et al., 2015). Similarly, HSP attenuated the AlCl₃-induced oxidative stress and cognitive deficits in the Alzheimer rat model (Justin Thenmozhi et al., 2017). Raza et al., (2011) demonstrated that HSP neuroprotective effects are attributed to its inhibitory effect on ROS generation, including hydroxyl and peroxynitrite radicals scavenging, and its mitigating effect on $Fe^{2+}$-induced linoleate peroxidation as well as cerebral membranes auto-oxidation.

## 5. CONCLUSIONS

Finally, these results demonstrate a novel applicability of hesperidin using chitosan-based nanogel to increase antioxidant response against aluminum and fluoride-induced brain oxidative stress suggesting it as a promising therapeutic approach for the prophylaxis and treatment of metals-induced brain toxicity.

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