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PROTECTIVE ROLE OF RESVERATROL AGAINST OXIDATIVE STRESS INDUCED BY ALUMINIUM AND FLUORIDE IN RAT BRAIN

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ABSTRACT

Aluminium and Fluoride, known to form a strong complex, are being used widely in many industries. Both elements are involved in the pathogenesis of neurodegenerative disorders by inducing oxidative stress. Many experimental studies reported neuroprotective properties of resveratrol (Res). In this study, we examined the ameliorative effects of resveratrol on aluminium fluoride-induced oxidative stress in the conventional and preclinical models. $AlCl_3$ (100 mg/kg bw) + NaF (10 mg/kg bw), $AlCl_3$ (100 mg/kg bw) + NaF (10 mg/kg bw) + Res (30 mg/kg bw), and Res (30 mg/kg bw) were administered for II, III and IV groups respectively and group-I was served as control for 8 weeks. The results showed considerable ($p<0.05$) alterations in protein content and oxidative markers (GPx, GST, GR, GSSH) in group-II whereas a significant ($p<0.05$) reversal was observed in group III. Taken together, the above findings indicate that resveratrol significantly alleviated the aluminium and fluoride-induced oxidative stress through its ameliorative efficacy.

KEY WORDS : Aluminium, Fluoride, Resveratrol, Oxidative stress, Neurodegeneration.

INTRODUCTION

Since decades, exposure to aluminium and fluoride is inevitable due to their abundance in nature and excessive usage in various industries (Kinawy and Al-Eidan, 2018). The principal sources of their entry into the body are contaminated air, aluminium and fluoride-containing medications, food and beverage packaging, drinking water and toothpaste etc (Chowdhury *et al*., 2016; Kinawy and Ezzat, 2013). Both elements can induce changes in the physiological processes (Benyettou *et al*., 2017) and it is well evident that Al and F are potent neurotoxic elements (Choi *et al*., 2015; Farhat *et al*., 2017; Ge *et al*., 2019). Aluminium capacity to cross the BBB increases in the presence of fluoride (Varner *et al*., 1998). These elements can cross the BBB and retain in the brain tissues, thereby, inducing oxidative stress, which alters antioxidant enzymes such as glutathione peroxidase, glutathione, glutathione reductase, glutathione-s-transferase activities (Julka and Gill, 1996; Vani and Reddy, 2000), and this would be more extensive in their combination (Kaur *et al*., 2009). This results in, changes in the antioxidant defence system causing prominent damage to the neural cells and alterations in the various brain regions (Ge *et al*., 2019; Nalagoni and Karnati, 2016; Varner *et al*., 1998) leading to neurodegenerative disorders (Narayanaswamy and Piler, 2010; Lu *et al*., 2017). Butterfield and Boyd-Kimball, (2005) reported a strong association between oxidative stress signs such as lipid peroxidation, protein content alterations, altered glutathione levels, reduced GST activity and neurodegenerative disorders such as Alzheimer's disease. In addition, glutathione paucity is associated with other neurodegenerative disorders such as Huntington's and Parkinson's diseases. Though neurotoxic mechanisms of aluminium and fluoride become clearer, more complete possible protectants against aluminium fluoride can be anticipated, which promises better outcomes in neurodegenerative disorders.

Resveratrol is a naturally occurring polyphenol compound (phytoalexin family) found in grapes, nuts, various berries, grape wines, pines, legumes as well as in the roots of the Japanese knotweed/ Itadori plant (Burns *et al*., 2002; Rimando *et al*., 2004),

and produced in response to injury or when the plant is under attack by pathogens such as bacteria or fungi (Langcake and Pryce, 1976; Soleas *et al*., 1997). The intense interest in the use of resveratrol is due to its Pleiotropic action as a molecule that affords protection against oxidative stress, inflammation, and as a caloric restriction mimetic (Baur and Sinclair, 2006; Cottart *et al*., 2014). It has received notable interest in the scientific and medical community as a possible treatment to combat several human chronic diseases (Baur and Sinclair, 2006). Over the last three decades, more than 2500 research articles have reported the health benefits of resveratrol and other stilbenes. These beneficial health effects include life-span extension, weight loss, protection against cardiovascular diseases, neurodegenerative diseases, stroke-induced brain damage, cancer, and cancer metastasis (Kasiotis *et al*., 2013). These investigations led us to look into the protective effects of resveratrol against Al and F induced neurodegeneration in rats. Thus, the purpose of the present investigation was to examine the protective efficacy of resveratrol against Al and F induced oxidative stress and it can be of great benefit to ameliorate the neurodegenerative disorders.

MATERIALS AND METHODS

Chemicals

$AlCl_3$, NaF and Res were procured from the Sigma Aldrich Company. The other chemicals used in the study were of analytical grade.

Animals and dosing

SD rats (body weight 180±10 g) were procured from the NIN, Hyderabad, TS, India. All the tests were carried out according to the departmental ethical committee (CPCSEA). The rats were maintained in the animal house in a microbial free environment at room temperature 24-26 °C for 1 week before the onset of experimentation for the adaptation to the laboratory. Standard rat chow and water ad libitum were given to the animals. The animals were categorized into four groups (five animals each). AlCl3+NaF (100 mg/kg bw+10 mg/kg bw), $AlCl_3$+NaF+Res (100 mg/kg bw+10 mg/kg bw+30 mg/kg bw), Res (30 mg/kg bw) were given to the Group-II, Group-III and Group-IV respectively. Group-I (control) animals received normal water. All the doses were given orally between 08:00 am – 09:00 am daily for eight weeks. For the biochemical analysis, the animals were sacrificed and the brains were taken out without delay for the study.

Protein estimation

Protein content was estimated by the method of Lowry *et al*., (1951). The colour intensity was read at 540nm. The result was expressed as mg protein/g weight of tissue.

Reduced glutathione (GSSH)

GSSH was measured by the method of Rehncrona *et al*., (2006). The rate of reduction of 5.5′-dithiobis-(2-nitrobenzoic acid) was measured at 412 nm. The amount of GSSH is expressed as n mole/mg protein.

Glutathione peroxidase (GPx)

The glutathione peroxidase activity was measured by the method of Martinez *et al*., (1979). The reaction was initiated with hydrogen peroxide (H_2O_2) and the oxidation of NADPH was monitored at 340 nm. The enzyme activity was expressed as units/mg protein.

Glutathione -s- transferase (GST)

The Glutathione-S-transferase activity was determined by the method of Habig *et al*., (1974). The increase in absorbance was recorded at 340 nm. The results were analyzed and expressed as µmoles/mg protein.

Glutathione Reductase (GR)

The GR activity was assayed according to the method of Beutler and Yeh, (1963). The reaction was started with the addition of oxidized glutathione and the change in absorbance was recorded at 340 nm. The result was expressed as unit/g.

Statistical analysis

The obtained data were analyzed statistically using one way ANOVA followed by Turkey's studentized range test. The significant level of $p<0.05$ was considered.

RESULTS

Protein Content

The protein content was significantly ($p<0.05$) decreased (22.84%) in $AlCl_3$+NaF treated group as compared to the control group. But, the protein content was significantly ($p<0.05$) recovered (16.16%) in Res+$AlCl_3$+NaF treated group as

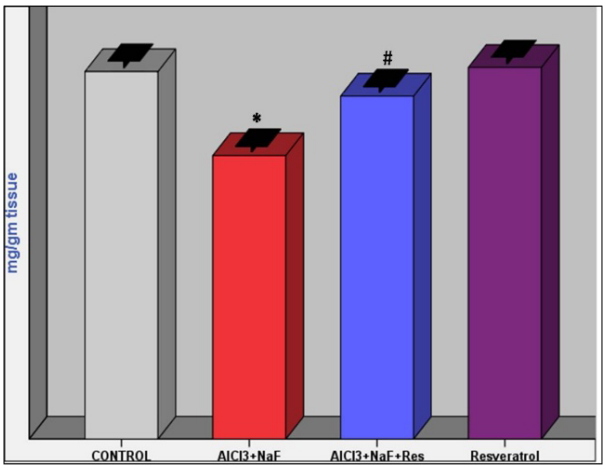

Fig. 1. Effect of Resveratrol treatment on protein content in rats subjected to $AlCl_3$+NaF treatment. The total protein content is expressed as mg/gm tissue. *P<0.05 as compared to the Control group and #P<0.05 as compared to $AlCl_3$+NaF treated group. Each value is mean ±S.E.

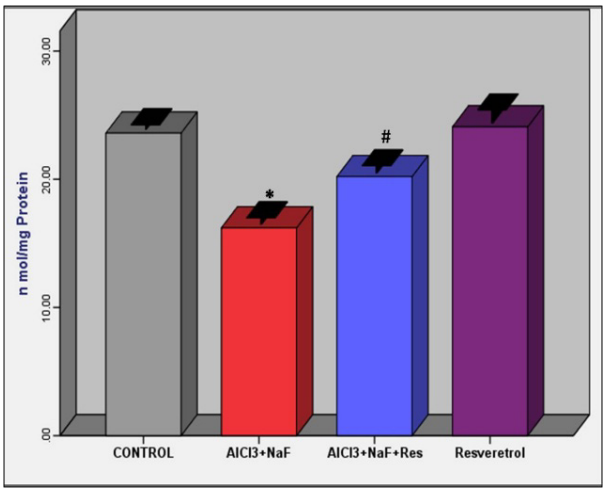

Fig. 2. Effect of Resveratrol treatment on GSSH in the cerebral cortex of rats subjected to $AlCl_3$+NaF treatment. The results are expressed as nmol/mg protein. *P <0.05 as compared to the Control group and #P<0.05 as compared to $AlCl_3$+NaF treated group. Each value is mean ±S.E.

compared to $AlCl_3$+NaF treated group. The group administered with Res alone showed insignificant variation as compared to the control group (Fig. 1)

Glutathione reduced

The Glutathione reduced (GSSH) was significantly (p<0.05) decreased (31.31%) in $AlCl_3$+NaF treated group as compared to the control group. But, the GSSH activity was significantly (p<0.05) reversed (16.95%) in Res+$AlCl_3$+NaF treated group as compared to the $AlCl_3$+NaF treated group. The Res alone administered group showed an insignificant difference as compared to the control group (Fig. 2).

Glutathione peroxidase

The Glutathione peroxidase activity was significantly (p<0.05) decreased (41.43%) in $AlCl_3$+NaF treated group as compared to the control group. But, the GPx activity was significantly (p<0.05) reversed (32.16%) in Res+$AlCl_3$+NaF treated group as compared to the AlCl3+NaF treated group. The group administered with Res alone showed an insignificant change as compared to the control group (Fig. 3).

Glutathione-S-Transferase

The Glutathione-S-transferase activity was significantly (p<0.05) depleted (22.59%) in $AlCl_3$+NaF treated group as compared to the control group. But, the GST activity was significantly (p<0.05) reversed (18.64%) in the group treated with

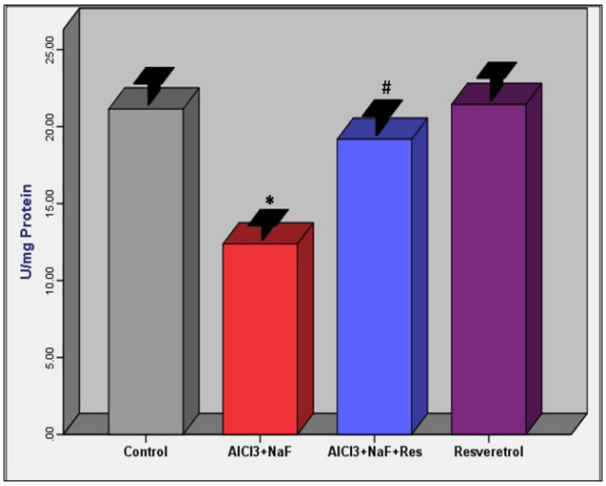

Fig. 3. Effect of Resveratrol treatment on GPx in the cerebral cortex of rats subjected to $AlCl_3$+NaF treatment. The results are expressed as U/mg protein. *P<0.05 as compared to the Control group and #P<0.05 as compared to $AlCl_3$+NaF treated group. Each value is mean ± S.E.

Res+$AlCl_3$+NaF as compared to the AlCl3+NaF treated group. The group administered with Res alone showed insignificant variation as compared to the control group (Fig. 4).

Glutathione reductase

The Glutathione reductase activity was significantly (p<0.05) decreased (21.72%) in $AlCl_3$+NaF treated group as compared to the control group. But, the GR activity was significantly (p<0.05) reversed (17.06%)

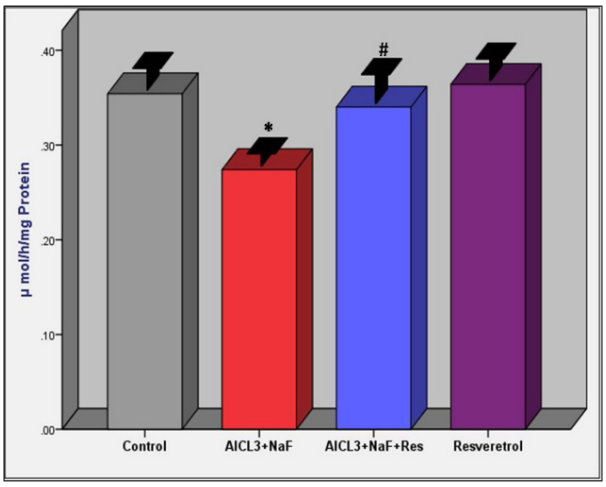

Fig. 4. Effect of Resveratrol treatment on GST in the cerebral cortex of rats subjected to $AlCl_3$+NaF treatment. The results are expressed as μmol/h/mg protein. *P<0.05 as compared to the Control group and #P<0.05 as compared to $AlCl_3$+NaF treated group. Each value is mean ± S.E.

in the Res+$AlCl_3$+NaF treated group as compared to the $AlCl_3$+NaF treated group. The Res alone administered group showed an insignificant difference as compared to the control group (Fig. 5).

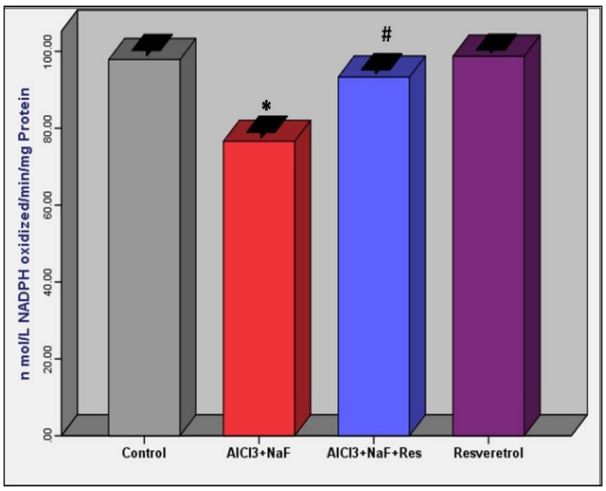

Fig. 5. Effect of Resveratrol treatment on GR in the cerebral cortex of rats subjected to $AlCl_3$+NaF treatment. The results are expressed as nmol/L NADPH oxidized/min/mg protein. *P<0.05 as compared to the Control group and #P<0.05 as compared to $AlCl_3$+NaF treated group. Each value is mean ± S.E.

DISCUSSION

Due to excellent Physico-chemical properties, Al and F are being massively used in several industries, which in turn lead to excessive exposure. Excessive exposure to Al and F may play a vital role in the aetiology of many diseases. Fluoride, silicon, iron, citrate, calcium etc affects aluminium absorption in human beings. Al and F have a high capability to enter the blood-brain barrier and remain in the brain and alters the cellular architecture leading to several disorders indicating their neurotoxic properties (Iqbal *et al*., 2016; Ge *et al*., 2019). Al and F damage the biomolecules such as lipids by generating reactive oxygen molecules which are a causative factor of oxidative stress (Wu *et al*., 2012). This investigation is reporting the antioxidant properties of Res against Al and F induced oxidative stress in the rat brain. Neurodegenerative disorders with oxidative stress are relatively explained by earlier studies (Banala *et al*., 2015; Mesram *et al*., 2017). It is known that both elements if excessively consumed, produces reactive oxygen species and thereby increase oxidative stress (Nalagoni and Karnati, 2016). In the present investigation, the significantly ($p<0.05$) decreased protein content and the activity of GST, GPx, GR and GSH in group-II in comparison with the control group indicates the increased oxidative stress and altered antioxidative defence system due to the production of free radicals corroborate the previous studies which reported the increased oxidative stress resulting in changes in the antioxidative defence system such as a decrease in the activity of Superoxide dismutase, Catalase, Glutathione-S-Transferase, Glutathione Reductase, Glutathione Peroxidase, Glutathione reduced and alterations in protein content (Butterfield and Boyd-Kimball, 2005; Pratico *et al*., 2002), leads to neuronal cell damage and various degenerative diseases (Narayanaswamy and Piler, 2010; Lu *et al*., 2017). Further, the changes in GST, GPx, GR and GSH leads to glutathione deficiency related to neurodegenerative disorders such as Parkinson's, Alzheimer's and Huntington's diseases (Nehru and Bhalla, 2006). Whereas, the altered antioxidant enzymes and protein content were significantly ($p<0.05$) reverted by the resveratrol. Res alone treatment did not show any negative results indicating its safety and efficacy. The protective properties of Res might be due to its antioxidative mechanism by scavenging the free radicals and restoring the antioxidative defence (Danli *et al*., 2019; Lech *et al*., 2018).

CONCLUSION

In conclusion, the present study reports the harmful effects of aluminium in association with fluoride in

the rat brain due to the free radicals production and thereby oxidative stress. The oxidative stress was significantly ameliorated by Res due to its effective role in the antioxidative defence mechanism by removing free radicals, restoring the activity of the antioxidant enzymes and inhibiting the damage to the neural tissues. Thus, resveratrol is said to be an effective neuroprotectant to prevent neuronal disorders. Further investigations are required to explicate a definite mechanism.

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Conflicts of interest

None.

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