Section A-Research paper

NEUROPROTECTIVE EFFECTS OF SYNTHETIC RESVERATROL ANALOGUE AGAINST FLUORIDE TOXICITY IN RAT BRAIN

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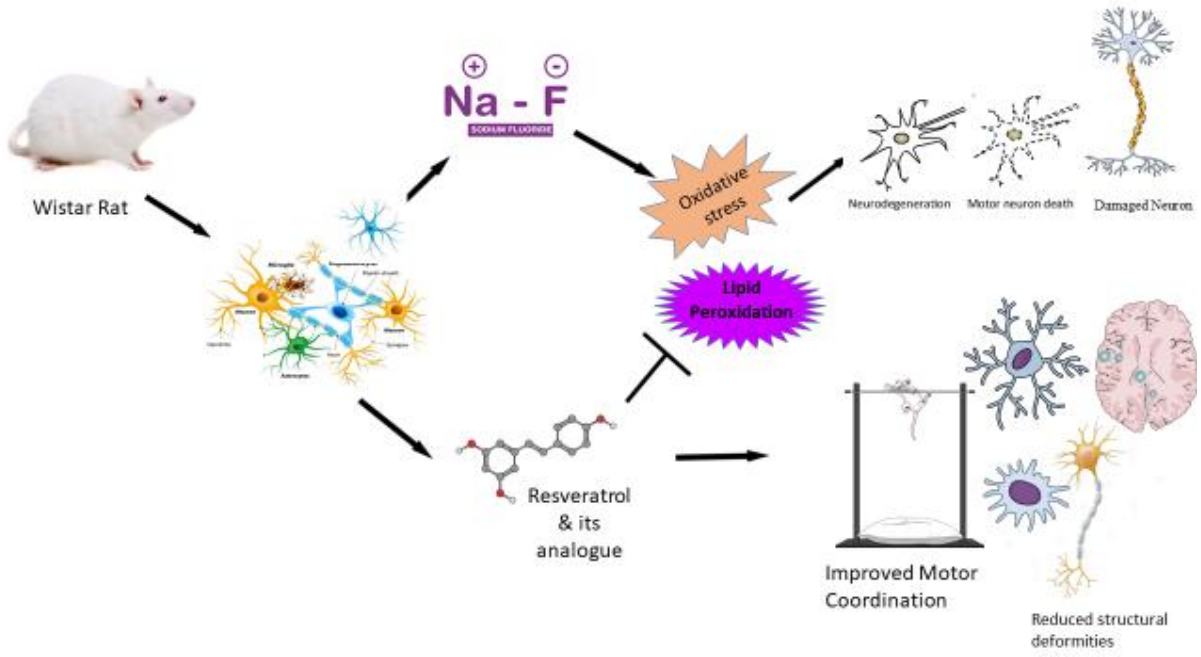
Abstract

Fluoride is a neurotoxic element that causes damage to neural tissue by crossing the cell membrane and blood-brain barrier. It induces oxidative stress and lipid peroxidation, resulting in neurodegeneration. Resveratrol (RSV) has been established to possess several beneficial properties, including antioxidant and anti-inflammatory effects. The present study aims to assess the neuroprotective effects of resveratrol and its synthesized analogue in Sodium Fluoride (NaF)-induced neurodegeneration in Wistar rats. The rats were given NaF, resveratrol, and its analogue for 14 days, and behavioural tests were conducted. Rats treated with resveratrol and its analogue exhibited increased motor coordination and decreased response time to external stimuli. Oxidative stress markers, lipid hydro-peroxide (LPO) and superoxide dismutase (SOD), were normalized in the treated groups. The antioxidant activity was found to increase dose-dependently. Histological analysis showed that resveratrol and its analogue reduced structural deformities in the brain tissue of rats treated with Sodium Fluoride. The degree of neuroprotection was significant in the groups treated with resveratrol and its analogue. Therefore, the synthesized analogue of resveratrol is a potent neuroprotective compound that could be an alternative to resveratrol due to its availability and ease of synthesis. This study highlights the importance of developing new therapeutic strategies for neurodegenerative diseases induced by fluoride intoxication.

Keywords Resveratrol · Resveratrol Analogue · Bioavailability · Neuroprotection · Fluoride · Oxidative Stress

Abbreviations RSV Resveratrol: NaF Sodium Fluoride: LPO lipid hydro-peroxide: SOD superoxide dismutase: bw body weight: GPx Glutathione peroxidase: CAT catalase activity: CA3 cornu ammonis: Analysis of Variance (ANOVA): COX-2 Cyclooxygenase-2.



Schematic representation of neuroprotective effects of synthetic resveratrol and it's analogue against fluoride toxicity in rat brain.

Introduction

Fluoride based on animal and human studies attributed as a neurotoxin. Exposure to fluoride can mainly occur through drinking water, respiration and intake of its supplements. At lower concentrations fluoride exhibit prophylactic effects (Chachra D et al. 2008). Prolonged exposure to fluoride accumulates in the body and create deleterious effects on dental, skeletal and soft tissues importantly like brain (Ge YM et al. 2005) and thyroid (Zhou B et al. 2007). Fluoride can cross cell membrane and blood brain barrier there by inducing toxicity and brings structural, metabolic and functional damages to the neural tissue (Yugandhar P Reddy et al. 2011). The main mechanism of fluoride lies in the excess generation of free radicals and lipid peroxidation thereby causing neuronal dysfunction and synaptic injury (Trivedi MH et al, 2007). Literature suggests that fluoride has massive destructive effects on neurotransmitters and anti-oxidative markers like vitamin C, SOD etc., triggering oxidative stress and also brings behavioural depression, diminished cognition and IQ (Nabavi SM et al. 2012; Bouayed J et al. 2010). Excessive activation of glutamate receptors by Fluoride is one other possible mechanism for its neurotoxic nature which is called as excitotoxicity. Fluoride can also create harmful effects in brain by inhibiting certain enzymes which are associated with energy production, membrane transport and synaptic transport (Lakshmi Vani M et al. 2000). Ultrastructural studies revealed that fluoride intoxicated animals showed neurodegeneration in cornu ammonis (CA3), CA4 and dentate gyrus regions of hippocampus which is mainly associated with cognition (Maheep Bhatnagar et al. 2002). Currently there is no cure for neurodegenerative diseases except managing the symptoms or halting the disease progression. Synthetic compounds and natural compounds are the two main sources of therapeutic strategies. Many natural plant products were discovered and were reported to be potential in reducing the neuro degenerative characters induced by NaF and other chemicals. Natural compounds like quercetin, curcumin, silymarin, arjunolic acid,

gallic acid were proved to have protective mechanism against NaF induced neurotoxicity (Costa LG et al. 2016; Monroy A et al. 2013; Marta Goschorska et al. 2017).

Resveratrol (trans 3, 5, 4-trihydroxystilbene) is an important polyphenol which recently gained the attention of research community. It is a dietary component found in berries, grapes and wine, exerts wide range of biological benefits (Wang Y et al, 2002). Resveratrol is well established to possess antioxidant, anti-inflammatory, anti-diabetic and anti-cancer properties (Lindsay G Carter et al. 2014). Oxidative stress is one important key factor in neuronal pathologies. Oxidative stress markers such as SOD, LPO, Glutathione peroxidase (GPx) and catalase activity (CAT) play vital role in neuronal degeneration in brain of rat. The neuroprotective property of these plant polyphenols seems to be mainly associated with its antioxidant activity. Resveratrol scavenges free radicals of reactive oxygen and nitrogen species by donating its hydrogen atom (Mohammed A Hussein 2011; Iuga C et al. 2012; Gerszon J et al. 2014). Free radical scavenging activity of resveratrol was studied in brain (Ates O et al. 2007) liver (Tunali-Akbay T et al. 2010) and kidney (Silan C et al. 2007). Resveratrol exerts its pharmacological effects through different mechanisms like involving in many signalling pathways, cellular mechanisms and antioxidant pathways. Resveratrol was also reported to have lifespan extending effects through activation of Sirtuins, a class of proteins which can triggers anti-apoptotic, anti-inflammatory and anti-stress responses (Outeiro TF et al. 2008; Gertz M et al. 2012) In spite of possessing such wide range of therapeutic properties, RSV and other polyphenols have few barricades like its low bioavailability which require its administration at higher doses, rapid metabolization in to less active metabolites hindering the therapeutic benefits *in vivo* (Foti Cuzzola V et al. 2011). These limitations shift the attention of researchers from natural to synthetic compounds and their analogues. Researchers from Centre for Plant Molecular Biology (Osmania University, Hyderabad) synthesised RSV analogue where they replaced one hydroxyl group by methyl group. The synthesis approach converted >99% of reactants into products with 97% purity. Anti-cancer activity of the synthesised compound was tested on HeLa and HEK293 cell lines and proved to have greater efficacy than resveratrol (Naini R et al. 2017) The RSV analogue was obtained from CPMB to evaluate its neuroprotective effects in Wistar rats. The main objective of this study is to evaluate neuroprotective role of synthesized compound, a resveratrol analogue against NaF induced oxidative stress, behavioural and histological alterations in rats. Behavioural tests, oxidative stress markers like SOD and LPO, free radical scavenging activity and histological studies were done to estimate analogue defensive effects against neurotoxicity.

Materials and Methods

Experimental design

Wistar rats of 1-2 months old weighing 60-70 gm were used for the study and were kept in standard lab conditions with a 12 h light/dark cycle and 24 ± 2°C temperature with food and water ad libitum. All experimental protocols were approved by the Ethics Committee of Department of Zoology, Osmania University (CPCSEA No: 383/01/a/CPCSEA). Resveratrol (purchased from sigma) and its analogue (obtained from CPMB) were dissolved in DMSO (< 5% was given to rats) and NaF was dissolved in water (**Fig 1**). Drugs were prepared freshly for each dose. It was synthesised in the laboratory of CPMB, using green chemistry by condensation reaction between aromatic 4-hydroxy aniline and 4-hydroxy, 3-methoxy benzaldehyde in *Psoralea corylifolia* hairy root extract. Healthy Wistar rats are divided into seven groups with six rats in each group. NaF, Resveratrol and its analogue were given intraperitoneal (IP) for 14 days. Group 1- control (physiological saline, IP); Group 2- NaF (30mg/kg bw, IP) (Krishnaiah C et al. 2007); Group 3- NaF (30mg/kg bw, IP) +

Resveratrol (50 mg/kg bw, IP) (Della-Morte D et al. 2009); Group 4- NaF (30mg/kg bw, IP) + Analogue (25 mg/kg bw, IP); Group 5- NaF (30mg/kg bw, IP) + Analogue (50 mg/kg bw, IP); Group 6- NaF (30mg/kg bw, IP) + Analogue (75 mg/kg bw, IP); Group 7- Only Analogue (75 mg/kg bw, IP). Resveratrol and its analogue were given after 30 min after the administration of NaF.

Behavioural studies

Rotarod test was conducted to study the noticeable behaviour by action, and action involves motor skills, including coordination of the body. Rotarod test is a conventional method to assess motor coordination and behaviour analysis, in which the animal is placed on horizontal rod which rotates (30 rpm) about its long axis (Hutter-Saunders JA et al. 2012). Before the experiment the rats were given training to run on the rod. On 14^{th} day the maximal time spent by rats on the rod rotating at 30 rpm were noticed to assess their motor coordination.

Hotplate test is used to evaluate the reflexes of animals to thermal pain. Delayed response to thermal pain indicates neural injury. During the experiment the rat was kept on hotplate instrument (Remi India, India) and the surface was maintained constantly at 52+0.50 ^{0}C. The time of exhibiting various behavioural responses like licking of forepaw and hind paw, rearing, brushing, jumping and withdrawal of paw from surface etc., were noticed as time to respond in seconds (Gunn et al. 2011).

The nociceptive withdrawal reflex is a defence mechanism to external stimuli was studied by Randall Selitto test. Changes in pain thresholds in different groups were determined by using a mechanical stimulus, the classic paw withdrawal test described by Randall and Selitto (Randall LO et al. 1957). A progressive increasing mechanical pressure was applied on each experimental rat on the dorsal surface of the paw using an analgesymeter and noted down the reads as paw withdrawal threshold in pounds.

Biochemical assays

The animals were sacrificed by cervical dislocation on 15th day and the whole brains were collected. Brain total Superoxide dismutase (SOD) levels were assayed by modified method of Marklund and Marklund, (1974) (Marklund S et al. 1974) 50% inhibition of autoxidation of pyrogallol by brain supernatant is calculated as one unit of SOD activity. Absorbance was measured at 420 nm using spectrophotometer.

The serum anti-oxidant activity of the rats dosed with resveratrol and its analogue were assessed through DPPH assay. Serum was deproteinized using equal volumes of acetonitrile (Chrzczanowicz J et al. 2008). DPPH was dissolved in methanol (10 mmol/L). The assay mixture comprises 970 µl of methanol, 5 µl of DPPH and 25 µl of deproteinized serum and the mixture was incubated for 30 min in dark before taking the readings at 517nm.

Malondialdehyde, the end product of lipid peroxidation was estimated in brain by using modified method of Ohkawa *et al*. (1979) (Ohkawa *et al*. 1979). The pink coloured tri-methine product absorbance was measured at 533 nm. The results were expressed as µmol of MDA/gm weight of tissue.

Histopathological assessment of brain

The animals were sacrificed on day 14 and brain tissues were fixed in 10% formalin. Paraffin embedded blocks was prepared after dehydrating the tissues in graded ethanol series. 5-10 micron sections were made on rotary microtome. Fine sections were used for H&E and Cresyl violet staining. Permanent slides were made and were used for evaluating histopathological changes under Olympus microscope (Lillie RD et al. 1976). In Golgi-Cox staining, the brain tissues were immersed in Golgi-Cox solution and kept in dark for 1 week. Sections were made using vibratome. Permanent slides were prepared for analysis (Anderson WJ et al. 1982).

Statistical Analysis

The samples were analysed in sextuplicate (Behavioural studies) and triplicates (Biochemical) and the results are presented as mean $\pm$ SD. One-way ANOVA was used for statistical analysis followed by student t-test. The p value of <0.05* was considered significant and p value of <0.001*** was considered highly significant.

Results

Body Weight

Weight gained by Fluoride treated rats is less relative to the control rats during the course of experiment. Weight gained by RSV and analogue treated groups is more than NaF group and less than control group. Group dosed with only analogue have equal increase in body weight (bw) to control group. However, the increase in body weights of RSV dosed rats is not much significant over NaF treated rats ($P>0.1$, NaF vs RSV). The mean increase in body weights of animals treated with RSV analogue at different concentrations are significant over NaF rats ($P<0.01$, NaF Vs Analogue treated groups). Treatment of rats with RSV and its analogue minimised the negative effects of NaF on body weights (**Fig 2**).

Behavioural tests

Rota rod

Motor co-ordination was very much decreased in fluoride treated rats over control rats ($P<0.001$, Control Vs NaF). All the treated groups showed significant endurance on rota rod over NaF treated group ($P<0.001$, NaF vs Treated groups). However, rats administered with Resveratrol (50 mg/kg bw) and analogue at higher concentration (75 mg/kg bw) demonstrated extremely significant performance. Group treated only with analogue (75 mg/kg bw) have equal effects to that of control (**Fig 3**).

Hot plate

Reduced heat sensitivity was observed in NaF group relative to control group ($p<0.001$, Control Vs NaF). Resveratrol and its analogue treated rats exhibited enhanced sensitivity towards heat ($p<0.01$, NaF Vs Treated groups). Rats treated with RSV+NaF, Analogue (75 mg/kg bw)+NaF and only with analogue(75 mg/kg bw) exhibited quick response to heat than rats treated with analogue at lower

doses (25 and 50 mg/kg bw). However, the time to respond was lessened in all the treated groups (**Fig 4)**.

Randall selitto

Hyperalgesia in fluoride administered rats is significantly greater than in control rats (P< 0.001). Nociceptive scores recorded lower for Resveratrol (50 mg/kg bw) and its analogue (75 mg/kg bw) treated groups than the groups treated at lower concentration i.e., at 25 and 50 mg/kg bw. Paw withdrawal threshold has been reduced in all the treated groups and it is much more substantial in RSV (50 mg/kg bw) and analogue (75 mg/kg bw) treated groups (p<0.01) (**Fig 5**).

Biochemical assays

Decreased levels of SOD were observed in fluoride administered rats (P<0.001, Control Vs NaF). The reduced levels of SOD have been recovered by resveratrol and its analogue significantly (P <0.001, NaF Vs Treated groups). Though rats dosed with Resveratrol (50 mg/kg bw), RSV analogue at 50 mg/kg bw and 75 mg/kg bw have shown equal effects on the expression levels of SOD which were decreased in NaF treated groups **(Fig. 6).**

As free radicals are directly involved in neurodegeneration, a significant level of free radicals of DPPH has been quenched by serum antioxidants of treated groups in an increasing order with increase in concentration (P<0.01, NaF Vs Treated groups). The percentage of scavenging free radicals is insignificant in NaF treated group. The groups treated with Resveratrol and analogue (75 mg/kg bw) exhibited nearly equal degree of activity (**Fig. 7**).

Lipid peroxidation levels were significantly high in NaF group compared to control group. The LPO levels reduced in a dose dependent fashion with the treatment of analogue which was significant over NaF dosed group (P <0.01, NaF Vs treated groups). Analogue at 75 mg/kg bw showed equal amount of activity to that of Resveratrol at 50 mg/kg bw (**Fig. 8**).

Histological studies

Healthy and round neuronal cells were observed in hippocampal and cortex region of control group rats. NaF treated group showed decreased number of cells, shrunken cells, irregular darkly stained cells and also decreased dendritic extensions. However, resveratrol and its analogue shielded the brain from the negative effects of NaF. The groups treated with RSV (50 mg/kg bw) and analogue (75 mg/kg bw) showed apparent normal appearance of cells similar to control group. There was a significant difference in cell morphology and neural network between NaF and treated groups. In addition, no harmful effects were noticed in analogue (75 mg/kg bw) alone treated group (**Fig. 9-11**).

Discussion

This study was carried out to know the protective effects of resveratrol and its analogue in NaF induced neurotoxicity in rat brain. It was well established that fluoride mainly acts through overproduction of reactive oxygen species causing oxidative stress and brain having large amounts of polyunsaturated fatty acids make it more vulnerable to oxidative stress (Shuhua X et al. 2012; Joels M 2008). It is clear from the results that resveratrol analogue is potent in antagonising the toxic effects of NaF in rats. The rats treated with RSV and its analogue at higher concentration (75 mg/kg

bw) did not show much increase in their body weights but still performed better in behavioural tests. Earlier it was reported that resveratrol regulates the body weight (Sharma R et al. 2017). Analogue at lower doses did not restrict the body weights but a higher dose limited the increase of body weight as resveratrol and it was not observed in the group treated only with analogue. The catechins present in green tea were reported exhibit anti-obesity effects through several mechanisms (Mohsen Meydani et al. 2010). It was also reported that increase intake of flavones, flavanols and catechins results in lower increase of body mass index associated with age in women (Hughes LA et al. 2008). Hence, we can assume that the RSV and its analogue might have its effect on body weights of rats. The behavioural studies like roto rod test, hot plate test and randall selitto test visibly show that all the treated rats i.e. either with resveratrol or its analogue at 25, 50 and 75 mg/kg bw reversed the impaired motor coordination and decreased the withdrawal latency period to thermal and mechanical pain than NaF treated rats. Although the synthesised resveratrol analogue at higher concentration (75 mg/kg bw) exhibited nearly equal effects as resveratrol at 50 mg/kg bw. Motor coordination was restored in RSV (oral dosage) treated rats against NaF and aluminium chloride ($AlCl_3$) in the studies done by Chandrashekar et al., from our lab (Chandrashekar et al. 2017). It is evident that berries contain resveratrol and in a study on berries improved the cognition and motor coordination in rats (Shukitt-Hale B et al. 2015) Dopamine and Dopaminergic neurons play an important role in the movement and motor coordination and dopamine levels get reduced on fluoride intoxication (Chouhan S et al. 2010). Oxidative stress has been implicated for dopaminergic neuronal cell death in Parkinson's disease. In previous studies it was shown that curcumin, naringenin and resveratrol upregulated the dopamine levels significantly in Parkinson's model rats and depressed mice (Zbarsky V et al. 2005; Gu Z et al. 2019). Fisetin is a polyphenol, was proved to prevent oxidative stress and neuroinflammation by inhibiting proinflammatory cytokines like interleukin (IL-6) and tumor necrosis factor alpha (TNF-α) which in turn downregulates Cyclooxygenase (COX-2), which is a potent mediator in inflammation (Sandireddy R et al. 2016) . Hence like polyphenols, resveratrol and synthetic RSV analogue might have neuroprotective effects by upregulating dopamine levels and downregulating proinflammatory cytokines.

The influence of oxidative stress induced by NaF and its setback by resveratrol and its analogue has been assessed by employing SOD, DPPH and LPO assays. In our lab Resveratrol and quercetin have been studied and were reported to have protective effects against NaF induced neurotoxicity (Nageshwar M et al. 2018). Resveratrol have protective effects not only against NaF but also to a wide variety of metals and chemicals like aluminium, manganese, streptozotocin, idarubicin, di-n-butylphthalate, 6-hydroxydopamine etc. RSV and the synthesised analogue were found effective in keeping the SOD levels nearer to control group either by increasing the expression levels of SOD (Kavas GO et al. 2013) or by lessening the burden on SOD from oxidative stress. Entry of a drug in to the blood stream is important to show its effects. Free radical scavenging activity of the compounds was estimated in the blood serum using DPPH assay and which indirectly explains the bioavailability of the drug. Groups treated with resveratrol and analogue at 50 and 75 mg/ kg bw almost had equal degree of free radical scavenging activity. Lipid peroxidation products are potential biomarkers to estimate the oxidative stress levels in neurodegeneration and other related diseases *in vivo* (Niki E 2008). We observed a significant reduction in the levels of MDA in resveratrol and analogue treated groups except at lesser dose when compared to NaF treated group. All the biochemical assays show that there is no negative effect in the rats treated only with synthesised compound. Histological studies clearly showed various degree of degeneration of neurons in rat brains. H&E images of the hippocampal region of the brain showed multi foci necrosis and apoptosis was observed in NaF treated group along with other degenerative features

like distorted cell shape, decreased cell density, demyelination and decrease in Purkinje cells. CA3 region of the hippocampus, which is associated with spatial learning and memory, was severely damaged with the fluoride intoxication as reported earlier (Tongjaroenbuangam W et al. 2011; Guan ZZ et al. 1998). Golgi cox staining is one of the best procedures for whole neuron visualisation and to study dendritic arborisation. Fluoride intoxication critically shrank the neural network. Decrease in cell density and Nissl substance was analysed by cresyl violet staining. All the damaging effects of fluoride were minimised by the administration of RSV and synthetic compound in a dose dependent fashion.

The deleterious effects of fluoride on brain were observed as reported earlier by Varner *et al*. in 1998 and Heba *et al*. in 2010 (Varner JA et al. 1998; Heba S et al. 2010). Resveratrol and its analogue succeeded in combating the oxidative stress induced damage by NaF. RSV analogue at 75 mg/kg bw had similar effects with resveratrol at 50 mg/kg bw. In vitro study conducted by Naini *et al*., 2017 on cancer cell lines showed that the synthesised analogue compound was more potent than RSV which is not observed in vivo study by us. Similarly, the NAD^+-dependent histone deacetylase SIRT1 was shown to be associated with aging and longevity (Naini R et al. 2019). RVS analogues reduced the oxidative stress by mediating several terminal kinases pathways like P38, JNK and ERK1/2 and also shown to exert cardioprotective and anti-aging effects by acting as modulators of Sirt1 (Raut et al. 2020). However, synthesis of RSV analogue compound is advantageous over synthesis of resveratrol, which involves multiple steps and release of toxic by-products.

Conclusion

In conclusion, the experimental data of this study clearly shows behavioural, oxidative stress markers and histological alteration induced with fluoride were reversed with Resveratrol and its synthesized analogue and analogue itself did not show any negative effects. In this study, RSV analogue at 75 mg/kg bw concentration has shown equal effects with Resveratrol at 50 mg/kg bw. Hence, Resveratrol analogue is suggested to be a potent neuroprotective compound which can be easily synthesised with less toxic by-products over Resveratrol and can be an alternative for Resveratrol in terms of its production and availability.

Statements and Declarations

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Conflict of Interest

The Authors declare that they have no Conflict of Interest.

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Legends to figures

Fig 1. Chemical structures of Resveratrol and its analogue

Fig. 2 Effect of resveratrol and Analogue treatment on mean body weight of rats subjected to NaF treatment. Weight gained by NaF group is very much less relative to control and RSV analogue treated groups. *p<0.001 against control, $^{\#}$p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in weight in gr.

Fig. 3 Effect of resveratrol and Analogue treatment on motor coordination (rotarod test) in rats subjected to fluoride treatment. Rats treated with NaF alone exhibited decreased motor coordination relative to all the other groups. RSV (50 mg/kg bw) and Analogue (75 mg/kg bw) almost nullified the effects of NaF on motor coordination. *p<0.001 against control, $^{\#}$p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in time in seconds.

Fig. 4 Effect of resveratrol and analogues treatment on latency period (hotplate test) in rats subjected to fluoride treatment. Decreased sensitivity towards heat was observed in NaF group. Latency period was reduced in all the treated groups relative to NaF group. *p<0.001 against control, #p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in time in seconds.

Fig. 5 Effect of resveratrol and analogues treatment on nociceptive pain (Randall selitto test) in rats subjected to sodium fluoride treatment. Paw withdrawal time was very much less in RSV and Analogue (75 mg/kg bw) treated groups relative to NaF group. *p<0.001 against control, #p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in withdrawal threshold

Fig. 6 Effect of resveratrol and analogues treatment on SOD activity in rats subjected to sodium fluoride treatment. SOD levels were significantly increased in RSV and analogue treated groups. *p<0.001 against control, # p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in present of inhibition

Fig. 7 Effect of resveratrol and analogues treatment on DPPH activity in rats subjected to sodium fluoride treatment. *p<0.001 against control, # p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in % of scavenging activity

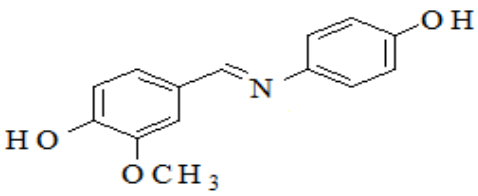
Fig. 8 Effect of resveratrol and analogues treatment on LPO content in rats subjected to sodium fluoride treatment. *p<0.001 against control, # p<0.01 against NaF. Data expressed as the Mean ± S.E.M (n = 6) and results shown in µmol of MDA / gm weight

Fig 9. Hippocampal region of rat brain in different groups. Blue arrow indicates necrosis in hippocampal region of rats treated with NaF. Yellow arrow indicates healthy cells in RSV and its analogue treated groups ameliorating the effects of NaF and also there is increase in cell density in analogue treated groups in a dose dependent fashion (Olympus microscope, 10X).

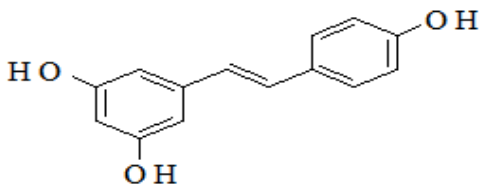
Fig 10. Golgi cox-stained sections of rat brain. Blue arrow indicates the damage of neural connections and network in NaF exposed rats. Yellow arrow indicates well branching of dendrites in RSV and analogue treated groups and the compounds reduced the deteriorative effects of NaF. Group treated with analogue at 75 mg/kg bw showed highest shielding effects (40X magnification).

Fig 11. Cortex region of different groups stained with cresyl violet. Protective effects of RSV and its analogue was observed against NaF showing round and healthy cells with cell membrane and clearly visible nissl granules which was indicated by yellow arrow. NaF treated group showed distorted and condensed cells undergoing necrosis with fewer nissl granules and was shown by black arrow (10X magnification).

Figures



RSV Analogue



RSV

Fig. 1

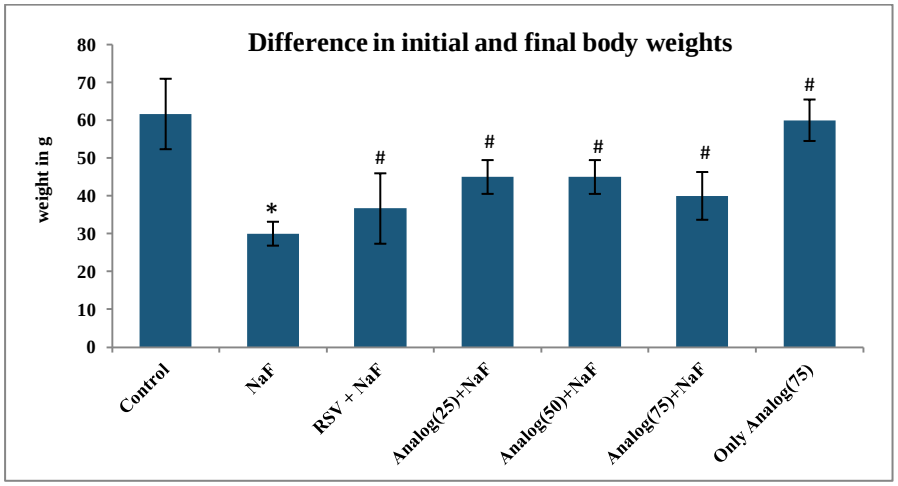
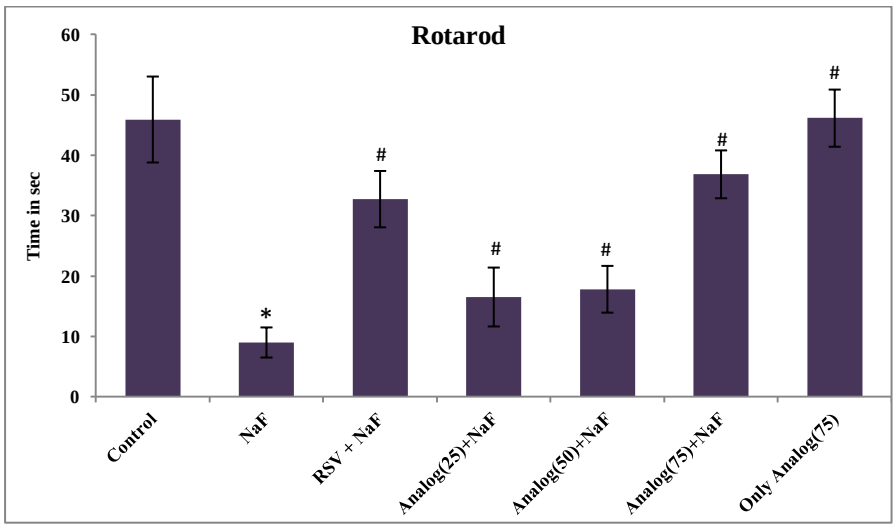


Fig. 2



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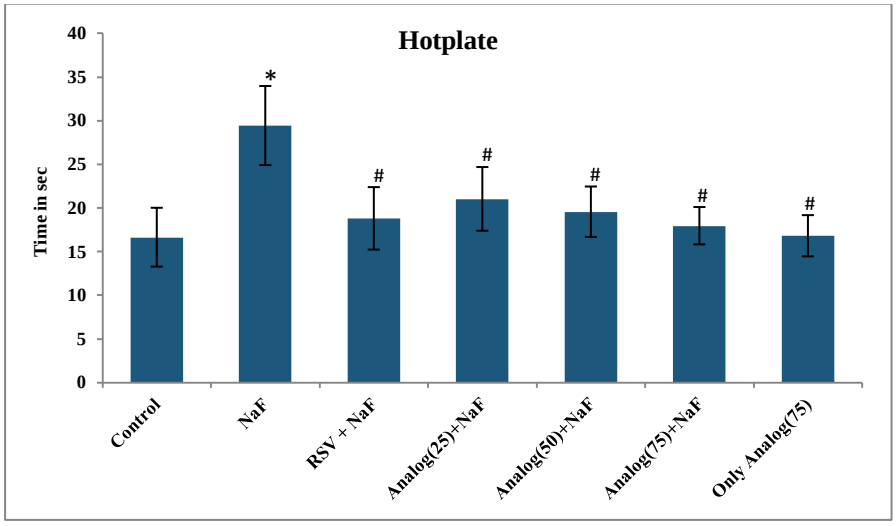

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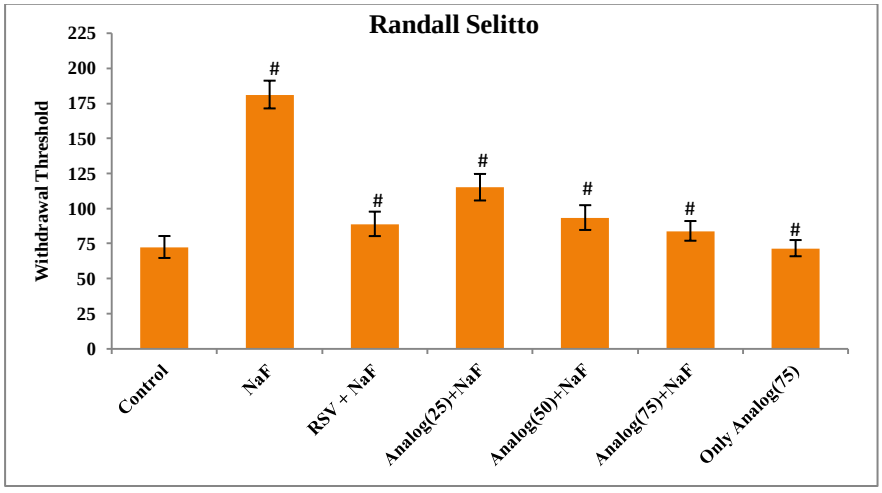

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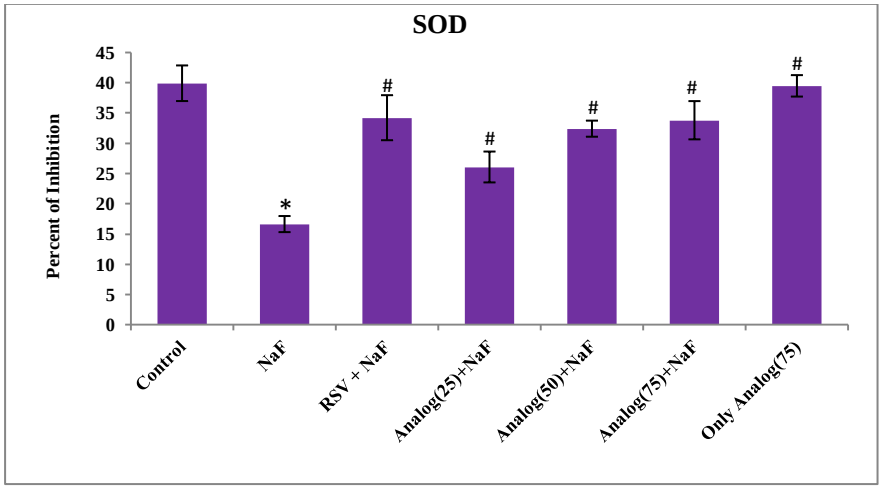

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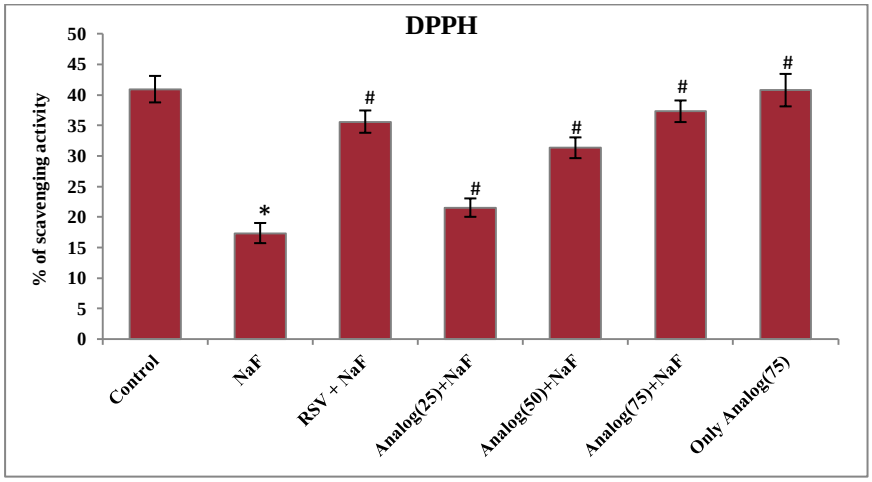

DPPH
% of scavenging activity
50
45
40
35
30
25
20
15
10
5
0
*
#
#
#
#
#
Control
NaF
RSV + NaF
Analog(25)+NaF
Analog(50)+NaF
Analog(75)+NaF
Only Analog(75)

Fig. 7

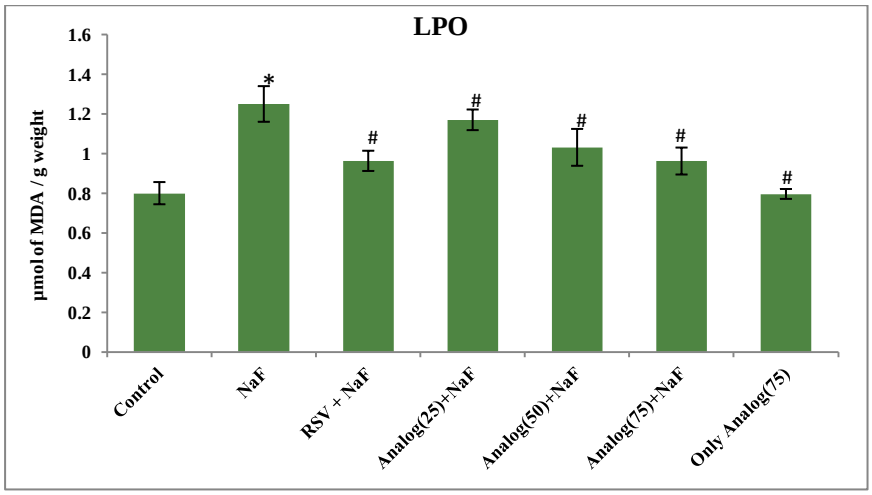
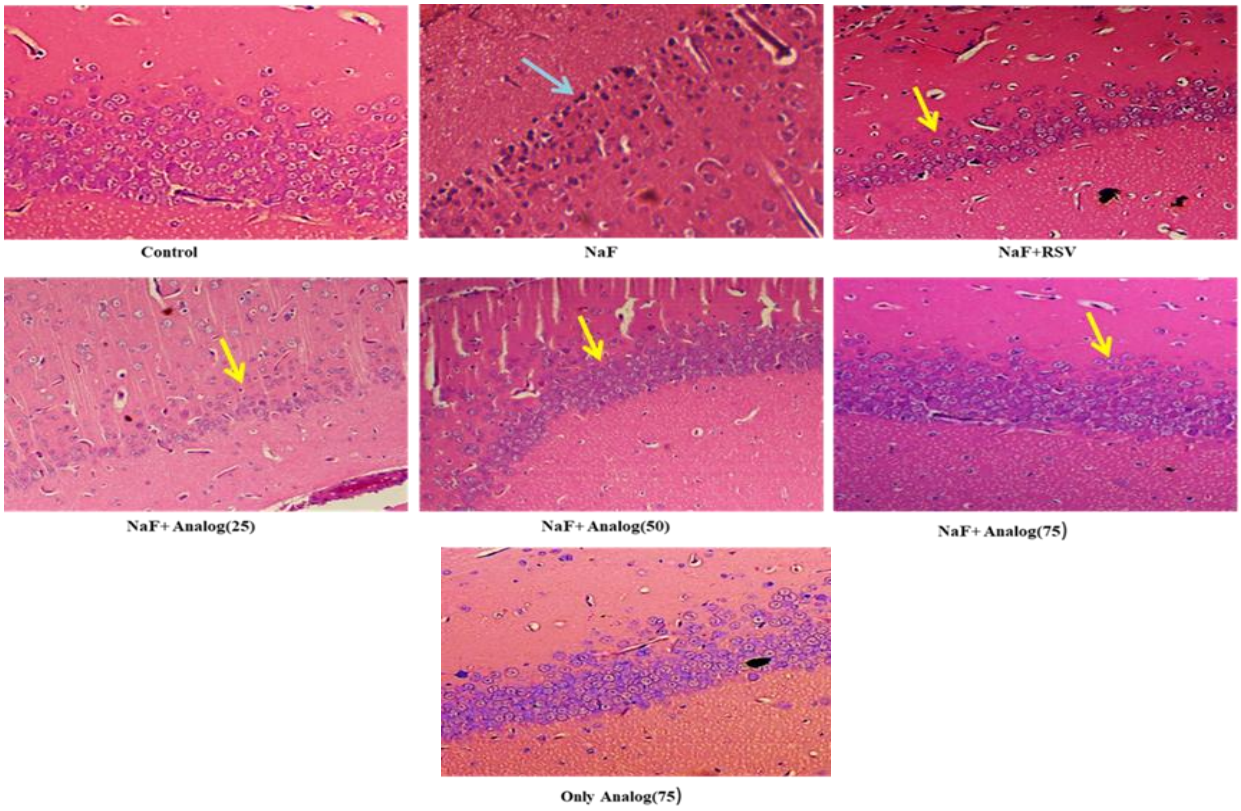

LPO
µmol of MDA / g weight
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
#
#
#
#
#
Control
NaF
RSV + NaF
Analog(25)+NaF
Analog(50)+NaF
Analog(75)+NaF
Only Analog(75)

Fig. 8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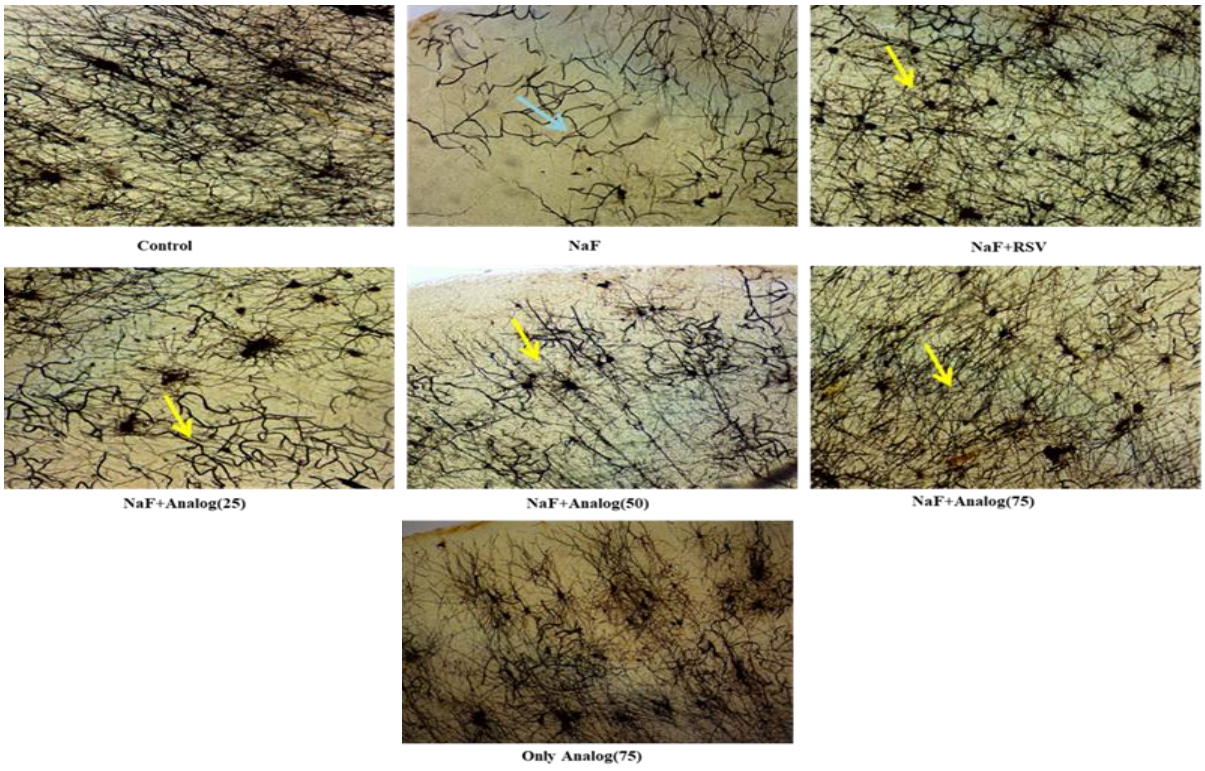
NaF+RSV
NaF+ Analog(25)
NaF+ Analog(50)
NaF+ Analog(75)
Only Analog(75)

Fig. 9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NaF+Analog(50)
NaF+Analog(75)
Only Analog(75)

Fig. 10

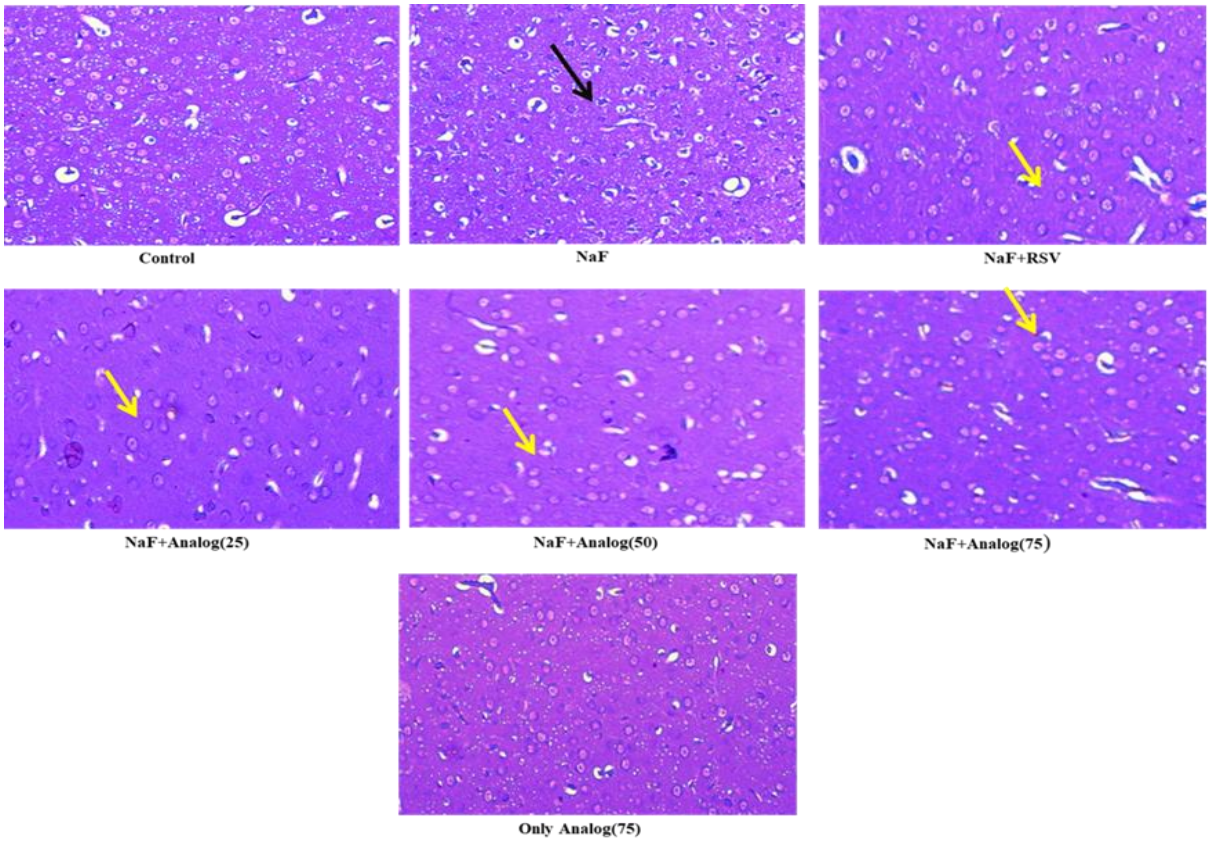
Control
NaF
NaF+RSV
NaF+Analog(25)
NaF+Analog(50)
NaF+Analog(75)
Only Analog(75)

Fig. 11