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Research Article

HINOKITIOL AND IMPERATORIN AS TYROSINE HYDROXYLASE INHIBITORS: A PILOT STUDY TO ANALYZE THE ANTI-PARKINSONISM POTENTIAL

Krishnapriya Madhu Varier, Sumathi Thangarajan *

Department of Medical Biochemistry, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai- 600113, INDIA.

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ABSTRACT

Parkinson's disease (PD), an age related disorder, owes the second most neurodegenerative disease in the world. A treatment regime for the disease is a need of the hour. One such from plant secondary metabolites may definitely owe for the drugs with reduced side effects. The adult zebrafishes administered by 6-Hydroxydopamine (6-OHDA) is used experimentally for mimicking Parkinsonism. Hinokitiol (HIN) also known as β-thujaplicin, has potential bioactivities like antibacterial, antifungal as well as antioxidant properties. Imperatorin (IMP) is a furanocoumarin utilized for several disease treatments. They are mainly available in plants like, Angelica dahurica and Angelica archangelica. Moreover, Amantadine (AMA), an aminoadamantane long known for its modest antiparkinsonian activity, has recently been shown to antagonize central nervous system dysfunction. In addition to mitochondrial damage and oxidative stress, the 6-OHDA, could result in reduced Tyrosine Hydroxylase (TH) levels in the cytosol. This is mostly held responsible for dopamine synthesis in the central nervous system, hence an antagonist of TH could be a potential drug of choice as anti-PD agent. Hence, in the present study, the comparative potentiality of Hinokitiol and Imperatorin as Tyrosine Hydroxylase inhibitors to analyze the molecular anti-TH interactions in 6-OHDA induced adult zebrafishes to alleviate Parkinsonism keeping Amantadine (AMA) as the standard drug.

KEYWORDS: *6- Hydroxydopamine, Hinokitiol, Imperatorin, Amantadine, Tyrosine Hydroxylase, Neurodegeneration.*

INTRODUCTION

Parkinson's disease marks the second position among neurodegenerative diseases reported in the world. This disease is regarded as an age- related condition as it is found majorly with persons beyond sixty years. In scientific terms, the disease characterizes the progressive loss of dopamine producing nerve cells in midbrain. More specifically, the affected region is substantia nigra of midbrain causing involuntary symptoms like tremor, rigidity, bradykinesia, and akinesia which shrinks the easiness of patients to undergo their daily routine. The worldwide occurrence of the disease is around 30% [1].

Hinokitiol (HIN), also known as β-thujaplicin, majorly found in the Heartwood of cupressaceous plants chelates iron [2] for a variety of biological processes. Moreover, the compound is recognized for its antibacterial [3], antifungal [4], as well as antioxidant capacities [5]. The compound by inhibiting the NF-κB activity and PLCg2 and/or PKC cascades normalizes the immune cell function and present antiplatelet activity [6]. Hinokitiol even impedes free radicals by controlling MAPKs and Akt activation. Hinokitiol even retain a neuroprotective property against thromboembolic stroke in rats [7]. Even though much study is there in the role of HIN in many signaling pathways, not many reports are there on its neuroprotective action in PD animal model.

Imperatorin (IMP) is a furanocoumarin utilized for several disease treatments. They are mainly available in plants like, *Angelica dahurica* and *Angelica archangelica* [8]. Wang et al., [9] 2013, reported that IMP by hypoxia re-oxygenation guards neuronal cells from apoptosis. They also showed that IMP enhanced cognitive deficiency induced by scopolamine [9]. AMA, an aminoadamantane long known for its modest antiparkinsonian activity, has recently been shown to antagonize central nervous system dysfunction. The PD symptom improvement with AMA may be of greater degree than improvement with other anticholinergic drugs, with less evidence of side-effects at optimal dosage levels. Moreover, AMA seems to have an additive therapeutic action with anticholinergic drugs [10]. Furthermore, the AMA may improve neuroleptic-induced tardive dyskinesia [11]. Thus, it is widely used in clinics as a treatment drug for PD symptoms and is thus used in this study as positive control.

*** Corresponding author:**

Sumathi Thangarajan

Department of Medical Biochemistry,
Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences,
University of Madras, Taramani Campus,
Chennai- 600113, INDIA.
** E-Mail: drsumathi.bioscience@gmail.com*

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The zebrafish is a tropical freshwater fish widely used now a days, as a premiere model organism to study neurological toxicity, especially 6-hydroxydopamine (6-OHDA) toxicity. The 6-OHDA administration to adult zebrafishes showed a loss of diencephalic dopaminergic neurons and resulted in unusual swimming pattern, illuminating phenotypic characters similar to PD patients. Thus, 6-OHDA administered zebrafishes could replicate a chemical induced hemi-PD model [12]. Moreover, 6-OHDA by inhibiting the activity of mitochondrial complex I, reduces cell viability and produces excessive Reactive Oxygen Species (ROS), a hallmark of the preliminary cellular signature of neurodegenerative diseases like PD [12].

In the substantia nigral portions due to 6-OHDA administration, the oxidative stress is found to be more [13], this in turn causes a reduction in the mitochondrial NADH dehydrogenase (complex I) activity [14, 15]. Thus the chief preliminary action of 6- OHDA toxicity is the uninterrupted mitochondrial respiration inhibition, tailed by enormous toxic events in cells like elevated peroxidation. It is considered that being a readily oxidizable compound, its by-products of its oxidation are free radicals, which could also elevate the toxicity process [16].

In addition, the intramuscular administration of 6-OHDA can even rise the expression of Tyrosine Hydroxylase (TH), a rate limiting step of dopamine synthesis [17]. In contrast, striatal 6-OHDA administration to rat brains, resulted in the projected rise in nigral dopaminergic neuronal death in substantia nigra [18] with reduced TH expression, which is due to TH-deficiency. The formation of L-DOPA is biochemically catalyzed by tyrosine hydroxylase (TH), the rate-limiting enzyme in the Dopamine synthesis, can be considered as the hallmarks of PD pathology. Thus, TH agonists, inhibitors of DA metabolism, could act as an effective tool for PD treatment [17]. The constant expression of this factor increases neurogenesis, synaptic plasticity, memory and learning [18]. Hence, the molecular interactions of HIN and IMP with TH and elevation in mitochondrial complexes and subsequent neurodegeneration were studied in 6-OHDA induced adult zebrafishes to alleviate PD keeping AMA as standard drug.

MATERIALS AND METHODS

Animals:

Wild type adult zebrafishes considered under this study were maintained in accordance Institutional Animal Ethics Committee (IAEC No: 01/01/2017) and the purpose of control and supervision of Experiments (CPCSEA), India, was followed by adherence to established protocols. Fishes were fed with commercial fish feed pellet. The water was changed regularly to maintain standard conditions. Groups of 6 adult fish of both sexes were housed at light/dark cycle of 14/10 in 10 liter water tank with continuous aeration and bio filtration. The water parameters like temperature, pH and conductance were maintained at 26-28^{0}C, 6.3-7.4, and 300 -480µS respectively.

PD induction:

Each group was employed with 6 adult fish of both sexes (1F:1M) per group. The fishes were anesthetized using 15 degree Celsius water and injected intraperitoneally with 6-OHDA at a dosage of 60 mg/Kg b.w of fishes dissolved in Ringer's solution (6.5g NaCl, 0.42g KCl, 0.25g $CaCl_2$ and 0.2g of sodium bicarbonate per liter) for 14 consecutive days for all the fishes, except the control group fishes, to induce PD, in accordance to [12] with slight modifications. An insulin syringe (U-40., 0.25mm (31 G) x 6mm needle) was used for intramuscular injections. The PD induction was done in the morning (9.00-10.00 a.m.) followed by oral administration of drugs (AMP, HIN and IMP) in the feed, after three hours.

Dosing of animals:

The AMA, HIN and IMP were dosed orally by mixing with the fish feed pellets, at fixed concentrations. Being the pioneer work on zebrafish models, the doses of all the three drugs were fixed after various pre-trails with varying concentrations. The AMA mixed food pellets served as positive control. The grouping of animal was done as follows.

Group 1: Control fishes administered with Ringers solution and pellets were coated with 0.01% DMSO + olive oil.

Group 2: Fishes were administered with 60 mg/kg b.w 6-OHDA intramuscularly (i.m) for 14 days [12].

Group 3: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 3mg/kg b.w of AMA orally on the same day for 14 days.

Group 4: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 10mg/kg b.w of AMA orally on the same day for 14 days.

Group 5: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 30mg/kg b.w of AMA orally on the same day for 14 days.

Group 6: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 3mg/kg b.w of HIN orally on the same day for 14 days.

Group 7: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 10mg/kg b.w of HIN orally on the same day for 14 days.

Group 8: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 30mg/kg b.w of HIN orally on the same day for 14 days.

Group 9: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 3 mg/kg b.w of IMP orally on the same day for 14 days.

Group 10: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 30mg/kg b.w of IMP orally on the same day for 14 days.

Group 11: Fishes were induced using 6-OHDA (60 mg/kg b.w) i.m and co-treated with of 60mg/kg b.w of IMP orally on the same day for 14 days. Fishes were fed, three pellets a day.

The compounds HIN, IMP and AMA, dosed for the study is mixed with fish pellets for oral administration. Each fish was fed separately.

Behavioral Analysis:

Behavioral tests, including Swim-Dive motion tests were designed according to the insights of [19]. Motor behavior test and anxiety behavior were analyzed by placing the fish in the aquarium and dividing the total height of the water zone into top 15cm and bottom 15cm. Fishes were allowed to acclimatize in measurement tanks for a period of 30 minutes before the readings were measured. Measurements were

recorded after on the 14th day, once the induction and the treatments were over and just prior to sacrifice for smear pathology. The number of the second fish spend on either zone was recorded for a period of 60 Sec using Mac CMOS 5MP motion capture lens for fishes.

Isolation of mitochondria:

After the experimental period of 14 days, the rats were sacrificed and the brain was removed by decapitation. The posterior tuberculin was pooled from each isolated brain and homogenized in the isolation buffer. Further, the tissue suspension was centrifuged for a period of 5 min at 13,000×g at 4°C. After centrifugation, the pellets formed were then eventually re-suspended in the isolation buffer that contains ethylene glycol tetra acetic acid (EGTA) and again allowed for centrifugation at 13,000×g for about 5 min at 4°C. The supernatants obtained were then transferred to fresh tubes and then topped off with the isolation buffer that contains EGTA and again allowed for centrifugation at 13,000×g for about 10 min at 4°C [20]. Finally, the obtained pellets that contain purified mitochondria were subsequently re-suspended in the isolation buffer without EGTA and were utilized for further analysis.

Measurement of mitochondrial complex activity:

Mitochondrial membrane bound enzyme activities of complexes I and IV were assessed spectrophotometrically in 100mmol/L of phosphate buffer with pH 7.4 at about 30°C. The activity of NADH-cytochrome *c* reductase was analyzed by the method of [21]. The change in absorbance was recorded spectrophotometrically at 550 nm for about 3 min and thereby the values obtained were denoted as units of NADH oxidized/min/mg of protein. The activity of cytochrome oxidase was assayed in accordance with [21]. The values were recorded and expressed as units of cytochrome c oxidized/min/mg of protein.

Histological analysis:

Fishes were anesthetized with 15 degree Celsius water and sacrificed by a cut between the brain and the spinal cord. Brain was dissected and portions of mid brain, including posterior tuberculum, portion were removed with a pin and a knife. The tissue was smeared on a glass slide and stained with *Haematoxylin & Eosin* for 2 minutes each followed by water washes. Slides were viewed at a 45X magnification (Olympus BX 53, Light microscope) and the number of degenerated neurons was counted using a hemocytometer for three fields per smear. Degenerated neurons were characterized by loss of cell structure, either swollen or constricted cells, irregular shaped cell membrane, stain relatively lighter with high rate of cell lysis during smear preparation [22].

q PCR analysis:

The total RNA was extracted from the posterior tuberculum of fishes by TRIzol according to the manufacturer's instructions and reverse-transcribed to cDNA (TaKaRa, Tokyo, Japan). The qRT-PCR was performed using the SYBR Premix ExTaq (TaKaRa, Tokyo, Japan) and mRNA-specific primers for tyrosine hydroxylase (FW- 5'- GCTGTGCTATGATTTCAGCACTT-3' RV- 5'- CGACAAAACTCCAAGCGCAA-3') were designed using the Primer 3 software. The relative mRNA level was normalized to GAPDH expression for each sample. Analysis of gene expression was carried out by the $2^{-\Delta\Delta Ct}$ method [23].

Statistical analysis:

Statistical analysis was performed and all the values are expressed as mean ± standard deviation (SD) and analyzed using one way analysis of variance (ANOVA) followed by Tukey's *Post-hoc* test (SPSS 20 version). Values with $p < 0.05$ were considered to be statistically significant.

RESULTS

Effect of IMP and HIN on 6-OHDA induced degeneration in the posterior tuberculum of control and experimental fishes:

The Table No. 1 shows the percentage of degenerative neurons in posterior tuberculum of control and experimental fishes. The current observation revealed that the neuronal regenerative capacity has been increased on administration of HIN when compared with IMP and AMA. The dosage of the AMA, HIN and IMP at 3mg/Kg b.w of 6-OHDA induced fishes has enhanced the neuronal regeneration up to 25% for the first two drugs and 10% for the latter. Whereas induced fishes treated with 10mg/Kg b.w of AMA and HIN regenerated the neurons up to 25% and 35 % respectively. At a dosage of 30 mg/Kg b.w of the AMA, HIN and IMP, treatment groups displayed different capacities of regeneration i.e., 45%, 35% and 20%, concurrently. Simultaneously, treatment of fishes with the highest dose of IMP at 60mg/Kg b.w has showed a neuronal regeneration of 20%.

Table No. 1: The Clinical parameters ensuring the degeneration as well as behavioral changes in control and experimental fishes.

Clinical parameters	Control	6-OHDA alone	Amantadine (mg/kg b.w)			Hinokitiol (mg/kg b.w)			Imperatorin (mg/kg b.w)		
			3	10	30	3	10	30	3	30	60
Percentage of degenerated Neurons	5	90	70	70	50	70	60	60	85	85	75
Motor behavior test	N	S	MS	MS	N	S	N	S	S	S	N
Anxiety Behavior test	N	A	MA	MA	N	A	N	A	A	A	N

Key Words: N – Normal; S - Slow; MS - Moderately Slow; A – Anxious; MA – Moderately Anxious

Histopathological Analysis:

The Histopathological observations have revealed that the posterior tubercular region of the induced fish brain had developed severe neuronal degeneration, drastic neuronal cell loss, increased inter neuronal space and glial cell loss with compared to control (Fig. 1 A&B). The histological disturbances had reduced on treatment with AMA in a dose dependent manner i.e 3, 10, 30 mg/Kg b.w. of fishes. The AMA at doses of 3 and 10 mg/Kg b.w resulted in severe cellular necrosis and inflammatory lesions (Fig. 1 C&D). Whereas 30 mg/Kg b.w of AMA regenerated both neuronal and glial cells (Fig. 1 E), when compared with induced fish brain cell pathology. Administration of HIN at the doses of 3 and 30 mg/Kg had shown severe pathological abnormalities like neuronal cell lysis, nuclear dislocation, cell shrinkage and inflammation, while at the dose of 30 mg/kg b.w. showed a good therapeutic effect indicating neuronal cellular regeneration, normalized nucleus and normalized glial cells (Fig. 1 F,G&H). Administration with

IMP had shown similar dose-dependency results with that of AMA indicating lower doses such as 3 mg/kg b.w. and 30 mg/kg b.w., showing pathological abnormalities and the higher dose of 60 mg/kg b.w. had shown a significant recovery in the neuron and glial cells along with normal architectural representation of the posterior tubercular region (Fig. 1 I, J&K).

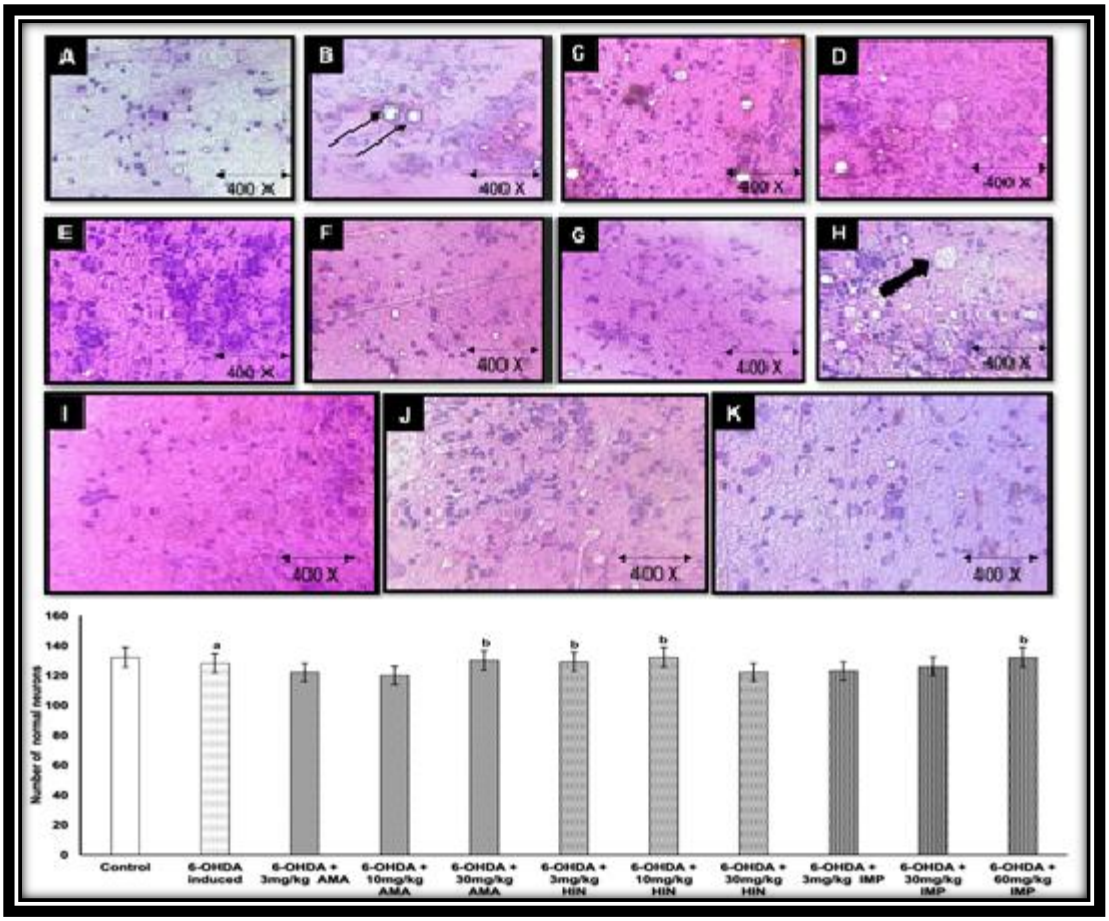

Fig. 1: Effect of IMP and HIN on 6-OHDA induced Histopathological alternations in the posterior tuberculum of control and experimental fishes

A) Control fishes, showing normal histological morphology of cells.

B) 6-OHDA alone, showing severe neuronal degeneration and drastic neuronal cell loss.

C) 3 mg/Kg b.w of fishes of AMA + 60mg/Kg b.w of fishes of 6-OHDA showing severe cellular necrosis.

D) 10 mg/Kg b.w of fishes of AMA + 60mg/Kg b.w of fishes of 6-OHDA, showing inflammatory lesions.

E) 30 mg/Kg b.w of fishes of AMA + 60mg/Kg b.w of fishes of 6-OHDA, showing regenerated neuronal cells.

F) 3 mg/Kg b.w of fishes of HIN + 60mg/Kg b.w of fishes of 6-OHDA, severe neuronal cell lysis, nuclear dislocation.

G) 10 mg/Kg b.w of fishes of HIN + 60mg/Kg b.w of fishes of 6-OHDA, showing better therapeutic neuronal plasticity.

H) 30 mg/Kg b.w of fishes of HIN + 60mg/Kg b.w of fishes of 6-OHDA, showing cell shrinkage and inflammation.

I) 3 mg/Kg b.w of fishes of IMP + 60mg/Kg b.w of fishes of 6-OHDA showing pathological abnormalities like vacuole development.

J) 30 mg/Kg b.w of fishes of IMP + 60mg/Kg b.w of fishes of 6-OHDA, depicting substantial nigral neuronal cell loss.

K) 60 mg/Kg b.w of fishes of IMP + 60mg/Kg b.w of fishes of 6-OHDA, showing significant recovery in the neuronal and glial cells loss.

Behavioral alterations analysis:

The behavioral pattern of zebrafishes assessed by motor behavior analysis on treatment with AMA, HIN and IMP at a dosage of 3, 10, 30 and 60 mg/Kg b.w of fishes improved its motor activities significantly ($P < 0.05$) when compared with 6-OHDA induced fishes (Table No. 1). But the fishes behaved normally as like control fishes at dosages of 30 mg/Kg b.w of AMA, 10 mg/Kg b.w of HIN and 60 mg/Kg b.w of IMP, respectively. Besides, comparable behaviors were observed in the anxiety motion analysis stating the therapeutic effectiveness of HIN at lower dosages in comparison to the IMP and positive standard.

Mitochondrial activity:

The mitochondrial activity of both the complexes 1 and 4 have significantly ($P < 0.05$) reduced when compared to 6-OHDA induced fishes (Fig. 2). The activity of the mitochondrial complexes 1 and 4 in both HIN and IMP treated fishes have increased significantly ($P<0.05$), when compared to control

mitochondrial activity. However, when the activities of both HIN and IMP were compared, HIN delivered better activities than IMP treatment at lower dosages.

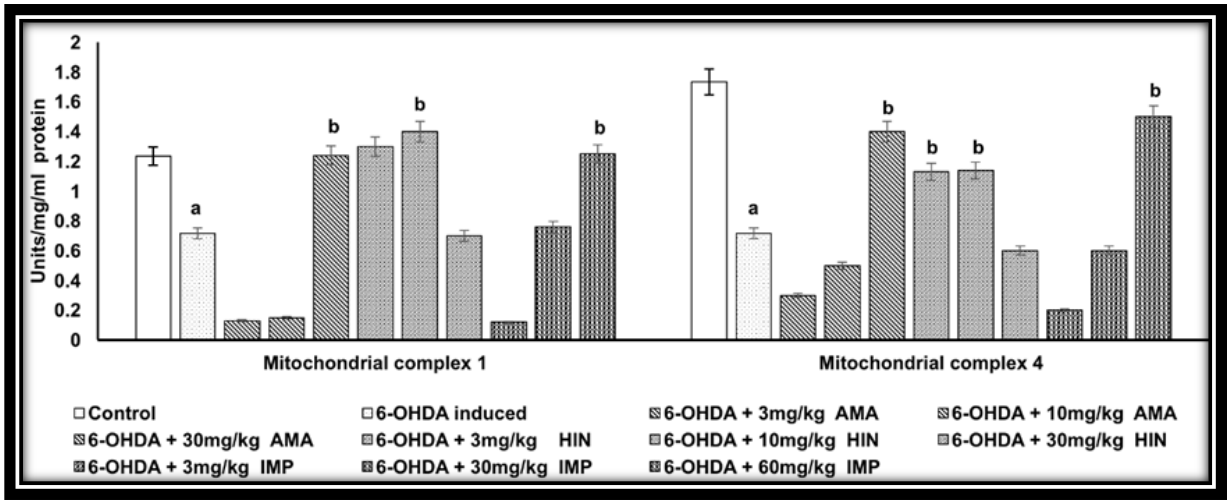

Fig. 2: Effect of IMP and HIN on 6-OHDA induced alternations in the activities of mitochondrial complexes 1 and 4 in posterior tuberculum of control and experimental fishes

Data represents mean ± SD. a. P < 0.05; b. P< 0.05; induced fishes were compared with control; Treatment groups were compared with 6-OHDA induced fishes by one way ANNOVA with turkey's post-hoc test.

Protein expression of Tyrosine hydroxylase

The tyrosine protein levels have significantly (P< 0.05) reduced in the 6-OHDA alone administered fishes when compared to the control fishes protein levels (Fig. 3). The HIN and IMP treatment significantly (P< 0.05) elevated the protein levels, which consequently resulted in the amelioration of 6-OHDA toxicity. Within HIN and IMP treatments, the 10mg/Kg dosage of HIN and 60mg/Kg of IMP executed more or less similar neuroprotection.

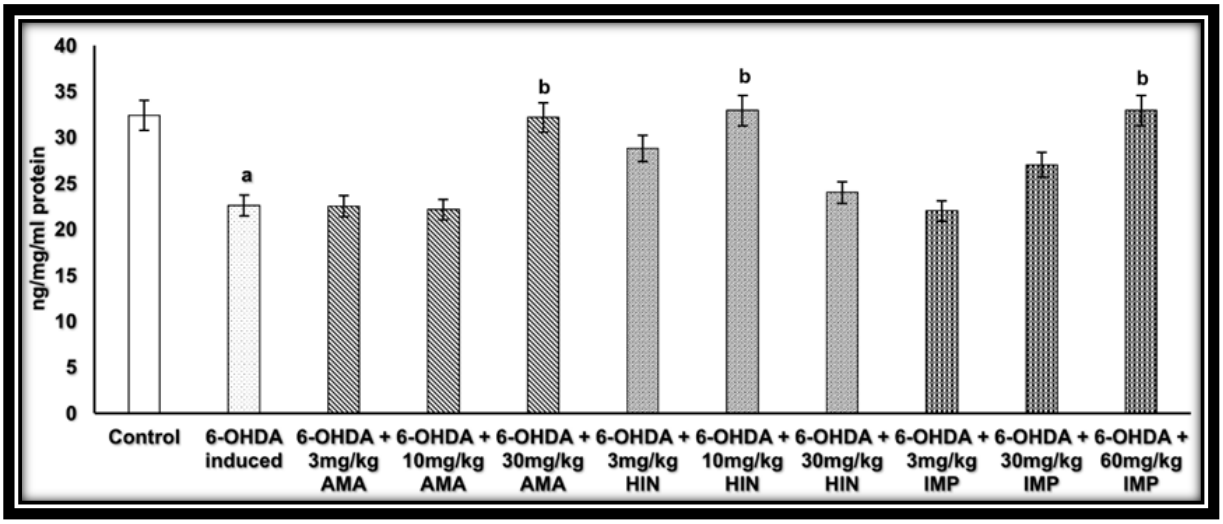

Fig. 3: Effect of IMP and HIN on 6-OHDA induced alternations in the protein expression of levels of Tyrosine hydroxylase in the posterior tuberculum of control and experimental fishes

Data represents mean ± SD. a. P < 0.05; b. P< 0.05; induced fishes were compared with control; Treatment groups were compared with 6-OHDA induced fishes by one way ANNOVA with turkey's post-hoc test.

The mRNA expression of Tyrosine hydroxylase:

The mRNA expression levels of Tyrosine hydroxylase in the 6-OHDA induced fishes were reduced significantly (P<0.05), when compared to control (Fig. 4). The treatment with a 10mg/Kg dosage of HIN and 60mg/Kg treatment of IMP apparently increased the expression levels in a significant (P<0.05) manner. On the contrary, when the dosages are comparing the minimal dose of 10mg/Kg dose of HIN could be preferred, as it advocated significant attenuation at minimal dose.

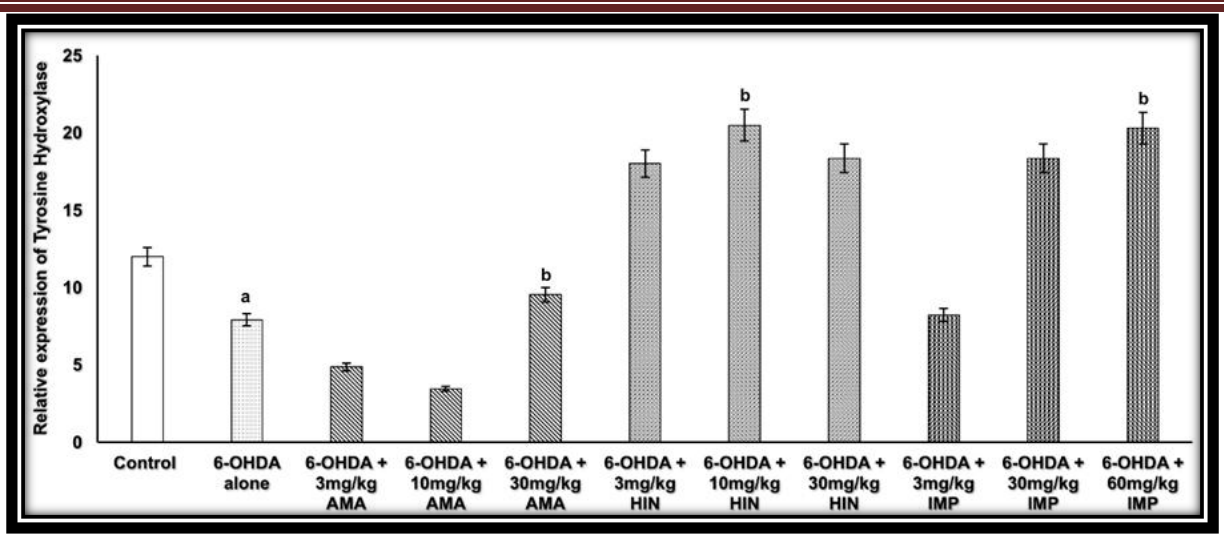

Fig. 4: Effect of IMP and HIN on 6-OHDA induced alternations in the mRNA expression of Tyrosine hydroxylase in the posterior tuberculum of control and experimental fishes

Data represents mean ± SD. a. P < 0.05; b. P< 0.05; induced fishes were compared with control; Treatment groups were compared with 6-OHDA induced fishes by one way ANNOVA with turkey's post-hoc test.

DISCUSSION

The selective neuronal loss in the substantia nigra (known as the posterior tuberculum in fishes) causing diminished dopamine levels acts to be the direct reason for neurodegeneration in Parkinsonian patients [24]. Furthermore, 6-OHDA, which is frequently administered compound for the induction of PD in experimental animals, is designated to facilitate dopaminergic cell demise [25]. Hinokitiol, a tropolone related compound isolated from cupressaceous plants employed neuronal protection in thrombic ischemic stroke rats [7]. Thus the neuroprotective potential of HIN on 6-OHDA-induced dopaminergic neuronal damage in adult zebrafishes was analyzed in this study.

Ever since, chordates have been recommended as the best models for illuminating neurodegenerative mechanisms [26]. Among such models, zebrafish have gained attention nowadays, in studies of drug therapies, as they are excellent models with respect to ease delivery regimes like simple addition to the water tank many a times. Likewise, microinjection techniques are now executed even within larval fish forms to deliver agents directly to specific cells/tissues [27]. Recent studies have emphasized on behavioral endpoints of zebrafish that reviews the basic neurological functions with respect to diverse types of sensory neurons as well as cognitive performance. Many behavioral endpoint tests investigate multiple neurological capabilities to link and evaluate specific perceptual, cognitive and physiological ailments. Moreover, several fish behavioral tests still remain in advancement. Most approaches developed recently have very strict analogous relationships with mammalian model's screening. Thus, in this study, swim and dive motion analysis were executed to explore the anxiety behavior and motor impairment, which is parallel to open field test and rotarod analysis in rats. Moreover, zebrafish movement had been established as a viable endpoint for perceiving neurological impairments during development. For instance, embryonic zebrafish had reduced swimming activity 6+ days after exposed to chlorpyrifos, an environmental pollutant [28]. Similarly, in our study, HIN treatment improved the swimming pattern of 6-OHDA administered fishes.

Several studies have demonstrated that the mitochondrial damage due to the inefficiency of their complexes like cytochrome oxidase/ mitochondrial enzymes results in PD progression. For instance the Parkin, PINK1, DJ-1 pathogenesis of PD occurs mainly due to mitochondrial membrane potential loss [29, 30]. In animal models of PD, it was being reported that the mitochondrial DNA (mtDNA) mutations in SN could eventually develop Parkinsonism [31]. Another study proclaimed the aptitude of 6-OHDA to hinder mitochondrial complex I and complex IV activities in the animal models of PD. Its inhibitory potency results in depleted mitochondrial respiration, causing ATP depletion and cellular degeneration [32]. Similarly, in our study there was a depletion in the activity of mitochondrial complexes 1 and 4 which were reverted back to normalcy via HIN treatment.

The 6-Hydroxydopamine also impedes TH, the rate-limiting enzyme in dopamine metabolism [18]. Thus, through above considerations, HIN treatment further elevated the levels of TH in substantia nigra of the brain owing to its therapeutic effect. Therefore, regulation of TH activity is very important, which can indirectly analyze the therapeutic efficiency of the drug of choice. The activity of TH can be regulated by two mechanisms: short-term direct regulation of enzyme activity and medium- to long-term regulation of gene expression [33, 34]. Thus, in this study as a pioneer analysis, the gene expression of the TH is being studied when orally administered with HIN and IMP. The mRNA expression results showed that the molecule can act as a potent enhancer of TH at therapeutic dosage, thus suggesting the ability of the drug to act as a potent ant-PD agent.

The zebrafish models are becoming a significant model for neuroscience, supporting their utility for pharmacological research. The oxidative mitochondrial stress responses, histopathological alterations, and behavioral abnormalities by the 6-OHDA administration were reverted back to normal by treatment with HIN, thus suggesting 10 mg/Kg as a therapeutic dose for Parkinsonism than of IMP. The mRNA level expression of TH also remained almost the same, suggesting, HIN as a potent anti-PD agent than IMP.

CONCLUSION

The study suggests that HIN can act as a better neuroprotective drug than IMP on 6-OHDA induced neurotoxicity through reducing the neuronal loss in the posterior tuberculum (similar to substantia nigra in mammals) of 6-OHDA administered adult zebrafishes. The study revealed that HIN was found to be a better therapeutic drug than IMP due to its neuroprotective efficacy at lower dose of 10 mg/Kg, which could be achieved by IMP only at 30 mg/Kg therapeutic dose. Further, the mRNA expression levels of Tyrosine Hydroxylase also followed the same therapeutic fashion. The decreased levels of TH genes upon 6-OHDA treatment were increased significantly ($P<0.05$) reduced in treated adult zebrafishes, suggesting HIN as a better anti-PD agent for 6-OHDA induced neurodegeneration.

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Ethics Approval:

All of the experiments were approved by the Institutional Animal Ethical Committee of the University of Madras (protocol no. IAEC No: 01/01/2017).

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