

Original Article

## 3-AMINOBENZAMIDE, A POLY (ADP-RIBOSE) POLYMERASE INHIBITOR, RESTORES BIOENERGETICS BUT FAILS TO ALLEVIATE EXCITOTOXICITY AND MOTOR FUNCTIONS IN 3-NITROPROPIONIC ACID INTOXICATED MICE

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### ABSTRACT

**Objective:** The present study was undertaken to investigate the effects of 3-aminobenzamide (3-AB), a poly (ADP-ribose) polymerase 1 (PARP1) inhibitor, on motor functions along with brain excitotoxicity and bioenergetics alterations in 3-nitropropionic acid (3-NPA) intoxicated mice model of Huntington's disease (HD).

**Methods:** Young healthy male C57BL/6J mice were pre-treated with vehicle/3-AB for a period of five days and intoxicated with two doses of 3-NPA (15 mg/kg, i. p) at 24 h interval on day 4 and 5. Animals were observed for motor functions 5 days after 3-NPA injection. They were sacrificed at the end of motor tests and brains were collected for neurochemical, bioenergetics, glial cells and cytokines analysis.

**Results:** 3-AB treatment significantly increased the bioenergetics (ATP and NAD) and succinate dehydrogenase activity in 3-NPA intoxicated mice brains. But, it failed to decrease glutamate content, cytokines-TNF $\alpha$  and IL-1 $\beta$  and glial markers–glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) expressions. Further, 3-AB administered produced only a non-significant restoration of motor functions in 3-NPA intoxicated mice.

**Conclusion:** The present study revealed that excitotoxicity and inflammatory pathways are major perpetrators in 3-NPA induced neurodegeneration and motor dysfunction. Therapeutic approach with 3-AB alone may not be sufficient to manage the multi-cascade pathogenetic mechanisms in HD neither symptomatic management too.

**Keywords:** 3-aminobenzamide, 3-nitropropionic acid, Excitotoxicity, Succinate dehydrogenase, Bioenergetics, Huntington's disease.

### INTRODUCTION

Huntington's disease (HD) is a neurodegenerative disorder, which affects mainly the basal ganglia in the brain. It is clinically characterized by a triad of motor dysfunctions, cognitive and psychiatric impairments. Though various biochemicals, molecular and pathological changes were extensively studied [1-3], the major targets in the pathogenesis of HD are not clear. Earlier reports have revealed that alterations in mitochondrial functions, in particular, succinate oxidation and energy depletion were observed in HD patients [4-6]. On the other hand, experimental reports also indicate the selective vulnerability of medium spiny neurons in neostriatum to glutamate excitotoxicity which reveals that excitotoxic neuronal death is also involved in HD [3].

PARP-1 is a nuclear enzyme and it is a double edged sword involved in cellular functions ranging from DNA repair to cell death. PARP-/- knockout mice showed decreased expression of inducible nitric oxide (*iNOS*) and TNF $\alpha$  triggered induced lipopolysaccharide (LPS) endotoxic shock [7]. This reveals that PARP1 propagates the necessary signal for cytokines generation. In addition, PARP1 inhibitors are shown to modulate brain inflammation through bioenergetics, *iNOS* and NF $\kappa$ B activity [8, 9]. Recent studies showed that 3-aminobenzamide (3-AB), a PARP inhibitor, exerts neuroprotection by directly inhibiting PARP and indirectly by anti-inflammatory and anti-apoptotic mechanism in cerebral stroke [10, 11]. Nam *et al.* [12] showed the involvement of oxidative stress and PARP1 activation in 3-NPA induced cytotoxicity in human neuroblastoma cells.

Though many evidences show the altered glutamatergic neurotransmission and energy deficiency come about parallelly in various neurodegenerative diseases, including HD, but no clear information on the predominant perpetrator [3, 13, 14]. Further, experimental animal models that mimic these clinical features in HD

are still a debate. Different animal models have been developed to study the vulnerability of striatal neurons to excitotoxicity which further triggers neuronal death. 3-nitropropionic acid (3-NPA), a neurotoxin, irreversibly inhibits succinate dehydrogenase leading to cell death [15]. Succinate oxidation by 3-NPA increases calcium influx, in turn *iNOS* stimulation and nitric oxide production, which sparks excitotoxicity and inflammation [16] and hence 3-NPA model was currently chosen.

The present study was undertaken to study whether therapeutic intervention with 3-AB, PARP1 inhibitor, alone can alleviate 3-NPA induced excitotoxicity, bioenergetics deficiency and finally the motor functions in mice model and can be considered for the symptomatic management of HD.

### MATERIALS AND METHODS

#### Chemicals and reagents

3-NPA and 3-AB were purchased from Sigma Aldrich, USA. Mouse anti GFAP was procured from Cell signalling, USA. Mouse anti IBA-1 and goat anti-mouse IgG were obtained from Santacruz Biotechnology, USA. BCIP/NBT substrate was procured from GeNei™, Mumbai. All other chemicals used were of analytical grade.

#### Animal husbandry and ethics approval

Male C57BL/6J mice (18-22g b. wt.) were used for the study. Animals were housed in groups (5 animals/cage) in polypropylene cages (air cycles: 12-15 air changes/h) under an ambient temperature of 22 $\pm$ 3 °C and 30-70% relative humidity, with a 12-h light/dark artificial photo cycle. They were provided with extruded rodent feed (M/s. Provimi Pvt, Ltd., India) and purified water, *ad libitum*. Guidelines of "Guide for the Care and Use of Laboratory Animals" were strictly followed. The protocol was approved by Institutional Animal Ethics Committee, Sri Ramachandra University, Chennai (IAEC/XXII/SRU/161/2011).

## Groups and treatment

Animals were acclimatized to the laboratory conditions for a period of 7 days. Following acclimatization, animals were grouped into 3 (10 animals in each) and pre-trained in beam walk test. Treatment was scheduled for five consecutive days. Group I and II received 0.5% carboxy methyl cellulose (CMC) and group III received 3-AB (20 mg/kg b. wt., i. p.) once daily. On day 4 and 5, Group II and III received 3-NPA (15 mg/kg b. wt., i. p.) half an hour after vehicle/3-AB administration. Animals were subjected to motor function assessment 5 days after 3-NPA injection, sacrificed and brain collected for further analysis.

## Motor function

### Beam walk experiment

Beam walk test was performed according to the Schallert method [17] with slight modifications. Mice were pre-trained before treatment, to traverse a narrow beam of 100 cm lengths to reach an enclosed escape platform. A bright light (20 lux) was placed above the narrow beam to create an aversive stimulus. This encourages the mice to traverse the beam to the dark enclosed goal box. The mice were placed individually at the start of the beam, 24 h after the last 3-NPA injection. The time taken by the mice to traverse from start of the beam to enclosed escape platform, number of foot slips and immobility period were recorded. The observer who scored the behavior of mice was blinded to the treatment groups.

### Horizontal grid test

Horizontal grid test was performed according to Kim *et al.* method [18] with slight modifications. Apparatus consisted of horizontal grid mesh (total size 12 cm<sup>2</sup>: openings 0.5 cm<sup>2</sup>) mounted 20 cm above a hard surface, thus discouraging falling but not leading to injury in the case of falling. Mice were placed individually in the centre of the horizontal grid and supported until it grabs the grid with both fore and hind paws. Grid was then inverted so that the mouse hangs upside down and they were monitored for hanging time and wall fault time. The observer who scored the behavior of mice was blinded to the treatment groups.

### Brain glutamate content

Animals were sacrificed following motor function test, brains were isolated, cortical, and striatal regions were micro-dissected and processed for glutamate estimation [19]. Brain homogenates were prepared in 0.1N HCl in 80% ethanol (for every 10 mg tissue/200 µl), centrifuged and supernatant was collected. Fresh supernatant was used for glutamate estimation using HPTLC (CAMAG-version 1.3.4, USA). Chromatographic conditions were as follows-stationary phase: silica gel GF254; mobile phase: n-butanol: glacial acetic acid: water (65:15:25, v/v/v); applicator: Linomat V; scanner: CAMAG TLC scanner III; developing chamber: twin trough glass chamber (20×10 cm); developing mode: ascending mode (multiple development-2 times); detection reagent: 0.2% ninhydrin in acetone; scanning wavelength: 486 nm and experimental condition: 25±2 °C temp/RH: 40-65%. Freshly prepared L-glutamic acid was used as standard and calibration curve was plotted with standard at 20-200 ng concentrations.

### Brain Succinate dehydrogenase (SDH) assay

Brain SDH activity was performed as previously described [20]. The tissue homogenate was diluted with 2.0 ml of cell extraction buffer. Assay mixture consists of 1.0 ml of 0.4 M potassium phosphate buffer (pH 7.2), 40 µl of 0.15 M sodium succinate (pH 7.0); 40 µl of 0.2 M sodium azide and 10 µl of 6.0 mg/ml 2,6-Dichlorophenol Indophenol (DCPIP). This mixture was used to obtain the baseline (zero) of the spectrophotometer reading at 600 nm. To 1.1 ml of this mixture, 200 µl of the sample was added. The rate of change in absorbance per minute was used to quantify the enzyme activity using the extinction co-efficient (19.1 MN<sup>-1</sup> cm<sup>-1</sup>) of DCPIP.

### Bioenergetics (NAD<sup>+</sup> and ATP content)

Cortical and striatal regions were processed for bioenergetics estimation. The specified brain regions were homogenized with 10%

KCl used for the estimation of NAD<sup>+</sup> and ATP level as described previously [21] with slight modifications. Homogenate aliquots were sonicated in ice-cold perchloric acid (0.1 N). After centrifugation (14,000 g, 4 °C, 5 min), 1 N sodium hydroxide were added to neutralise the supernatant. ATP/NAD<sup>+</sup> levels were determined using a reversed-phase high performance liquid chromatography (RP-HPLC) (Perkin Elmer, India).

### ELISA

Inflammatory markers such as TNF-α and IL-1β levels were measured following ELISA kit method (Ebioscience, Inc. USA).

### Immunoblotting

Striatal and cortical tissue samples were homogenised in 0.1 M ice cold Tris hydrochloric acid (pH 7.4) and centrifuged at 3500 rpm for 10 min. Protein concentrations of the supernatant were measured using Bradford reagent [22]. Aliquots containing 10 µg total proteins per sample were mixed with sample loading dye (β-mercaptoethanol and bromophenol blue) and electro phoresed on 12% sodium dodecyl sulphate polyacrylamide gel (Hoefer, USA). Separated proteins were transferred to polyvinylidene difluoride membranes, which were blocked overnight in 3% bovine serum albumin in Tris-buffered saline. Membranes were then washed thrice for 5 min each in Tris buffered saline and probed with primary antibodies. After three 5 min washes in TBS, membranes were probed with secondary antibodies for 1 h then washed again in TBS and incubated in BCIP/NBT substrate for alkaline phosphatase kit for 10 min. Bands obtained were visualised and semi-quantified using Bio1D software in gel documentation (Vilber Lourmat, France). The following primary antibodies were used: mouse anti GFAP (1:500) and mouse anti IBA-1 (1:200). Goat anti-mouse IgG (1:1000) was used as the secondary antibody.

### Statistical analysis

Data was expressed as mean±standard error means (SEM). Mean difference between groups were analysed by one way ANOVA followed by Tukey's multiple comparison as post hoc test using Graph Pad Prism 5.0 (San Diego, USA) software. p value ≤ 0.05 was considered significant.

## RESULTS

### Effect of 3-AB on motor functions in 3-NPA intoxicated mice

#### Beam Walk Experiment

3-NPA mice took longer time (p<0.01) to cross the marked zone and increased immobility period (p<0.01) and number of foot slips (p<0.01) when compared to the vehicle treated normal mice. 3-AB administration showed a non-significant decrease in time taken to cross the marked zone, immobility period and number of foot slips when compared to 3-NPA intoxicated mice (fig. 1).

#### Horizontal grid test

3-NPA mice showed the significant decrease (p<0.01) in hang time when compared to the vehicle treated normal mice. 3-AB administration showed no significant difference in hang time in comparison to 3-NPA intoxicated mice (fig. 2).

### Effect of 3-AB on brain glutamate content in 3-NPA intoxicated mice

A significant increase in glutamate content was observed in striatal (p<0.01) and cortical (p<0.05) brain regions of 3-NPA intoxicated mice when compared to vehicle treated normal mice. 3-AB administration showed no significant difference in glutamate content when compared to 3-NPA mice brain (fig. 3).

### Effect of 3-AB on brain succinate dehydrogenase activity in 3-NPA intoxicated mice

A significant decrease (p<0.01) in succinate dehydrogenase activity was observed in striatal and cortical brain regions of 3-NPA intoxicated mice when compared to vehicle treated normal mice. Pre-treatment with 3-AB significantly (p<0.01) increased the activity in comparison to 3-NPA mice brain (fig. 4).

**Effect of 3-AB on bioenergetics in 3-NPA intoxicated mice**

A significant decrease ( $p < 0.01$ ) in striatal ATP and NAD<sup>+</sup> contents was observed in 3-NPA mice brain when compared to vehicle treated normal mice. 3-AB administration significantly ( $p < 0.01$ ) increased the striatal ATP and NAD<sup>+</sup> contents in comparison to 3-NPA mice brain (fig. 5). There was no difference in cortical ATP and NAD<sup>+</sup> contents between the groups.

**Effect of 3-AB on inflammatory markers in 3-NPA intoxicated mice**

A significant increase in TNF $\alpha$  ( $p < 0.01$ ) and IL-1 $\beta$  ( $p < 0.05$ ) levels were observed in striatal and cortical brain regions of 3-NPA mice when compared to vehicle treated normal mice. There was no significant decrease in striatal and cortical TNF $\alpha$  and IL-1 $\beta$  levels in 3-AB administration were observed when compared to 3-NPA mice (fig. 6)

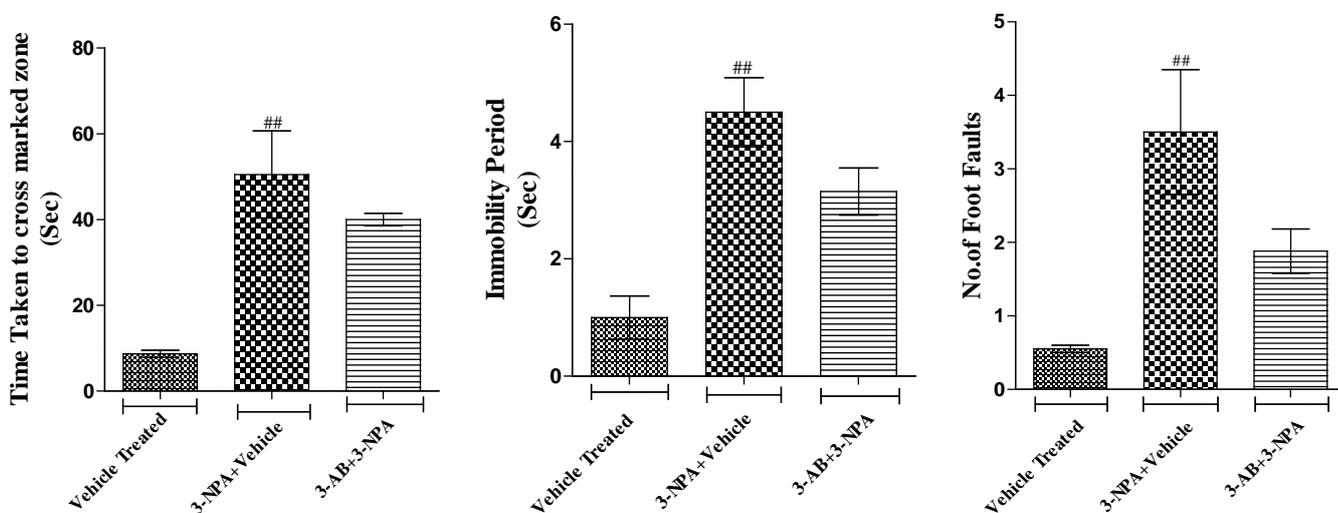


Fig. 1: Effect of 3-AB on beam walk experiment. Values were expressed in mean $\pm$ SEM; n=10 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.01 Vs vehicle treated group

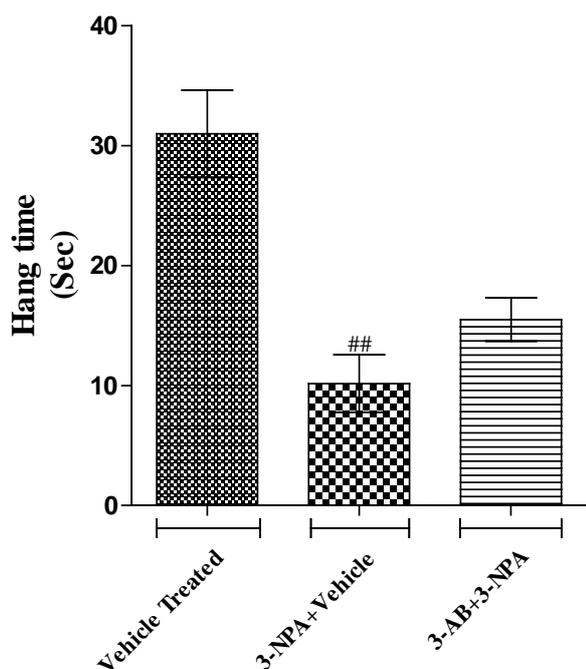


Fig. 2: Effect of 3-AB on horizontal grid test. Values were expressed in mean $\pm$ SEM; n=10 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.01 Vs vehicle treated group

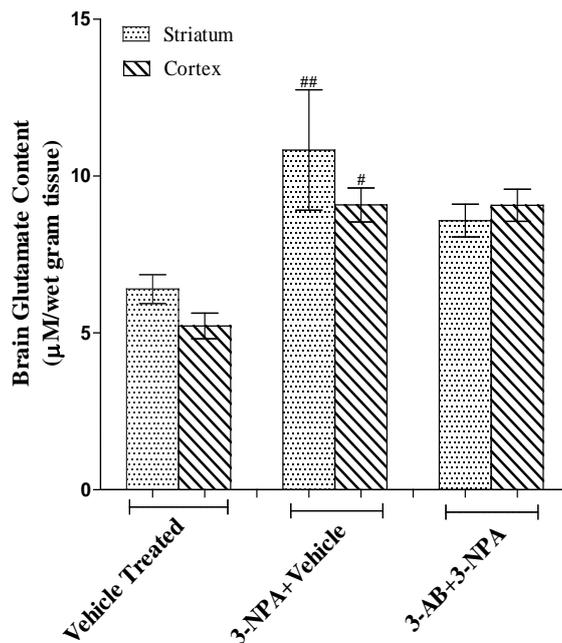
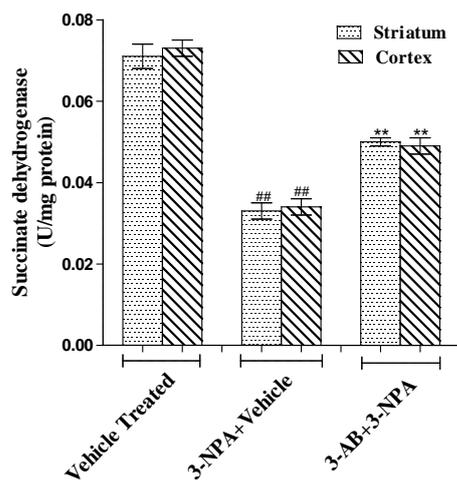


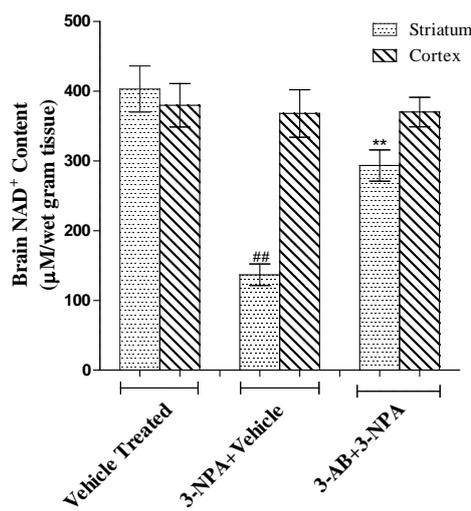
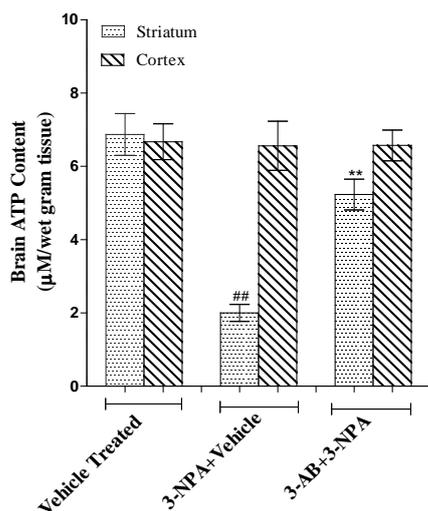
Fig. 3: Effect of 3-AB on glutamate content in 3-NPA intoxicated mice brain. Values were expressed in mean $\pm$ SEM; n=3 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.05 and 0.01, respectively Vs vehicle treated group



**Fig. 4: Effect of 3-AB on succinate dehydrogenase activity in 3-NPA intoxicated mice brain. Values were expressed in mean±SEM; n=3 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.01 Vs vehicle treated group; \*\* indicates p value<0.01 Vs 3-NPA+Vehicle treated group**

#### Effect of 3-AB on glial markers in 3-NPA intoxicated mice

A significant increase ( $p < 0.01$ ) in GFAP and IBA-1 expressions were observed in striatal and cortical brain regions of 3-NPA mice when compared to vehicle treated normal mice. Pre-treatment with 3-AB showed no significant difference in GFAP and IBA-1 expressions when compared to 3-NPA mice (fig. 7).



**Fig. 5: Effect of 3-AB on bioenergetics (ATP and NAD<sup>+</sup> levels) in 3-NPA intoxicated mice brain. Values were expressed in mean±SEM; n=3 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.01 Vs vehicle treated group; \*\* indicates p value<0.01 Vs 3-NPA+Vehicle treated group**

Another vital component that contributes to the vulnerability of striatal neurons to excitotoxicity is the surrounding glial cells [33]. Activation of glial cells in turn stimulates the inflammatory processes. Intoxication with 3-NPA was shown to up-regulate GFAP and IBA1 expressions in experimental animal model [34]. Recently, we have shown a strong positive correlation between the glutamate content and GFAP expression in 3-NPA mice model of HD. In the present study, 3-AB failed to down-regulate GFAP

#### DISCUSSION

Impaired mitochondrial functions through complex II inhibition, altered bioenergetics and increased excitotoxicity exacerbates the progression of neurodegeneration in HD [23]. In the present study, 3-AB increased succinate dehydrogenase activity and bioenergetics ATP and NAD<sup>+</sup> contents in 3-NPA intoxicated mice brain. However, 3-AB failed to restore glutamate excitotoxicity and inflammatory events.

It is well known that mitochondria play a key role in cell survival by regulating energy metabolism, apoptosis and calcium homeostasis [24, 25]. Reduction of mitochondrial complex II/III activity was observed in caudate and putamen brain regions of HD patients [26]. Furthermore, cultured striatal neurons injected with huntingtin (Htt) protein revealed a marked decline in complex II/succinate dehydrogenase activity which precedes energy depletion and neuronal cell death [27]. Estrada Sánchez *et al.* [3] showed that exposure to 3-NPA generated metabolic inhibition and mitochondrial dysfunctions that were shown to be due to oxidative stress, DNA strand break and PARP activation in human neuroblastoma cells. The increased succinate dehydrogenase activity and bioenergetics contents (ATP and NAD<sup>+</sup>) in the present study revealed that 3-AB to suppress the cellular consumption of ATP and NAD<sup>+</sup> through direct inhibition of over-activated PARP1.

Various studies demonstrated that NMDA antagonist, MK-801 and memantine exert protection against striatal lesions by suppressing excitotoxicity and improving succinate dehydrogenase activity in 3-NPA mice model [28, 29]. It has been shown that the glutaminergic system contributes to the neuronal cell death by NMDA receptor activation with excitotoxicity in HD [30, 31]. PPAR inhibition by 3-AB, decreased injury after focal ischemia in rats through reducing NMDA induced glutamate efflux and neurotransmitter dysfunction [32]. In the present study, pre-treatment with 3-AB did not showed any effect on glutamate level, i. e. excitotoxicity. This reveals meager restoration of bioenergetics may not alleviate excitotoxicity either directly or indirectly.

and IBA1 and also the cytokines TNF $\alpha$  and IL-1 $\beta$  levels in 3-NPA intoxicated mice which could be speculated to failure to control excitotoxicity. Further, alleviation of excitotoxicity was seemed to be more essential than bioenergetics restoring motor function in HD. Also, striatal region was found to be more vulnerable to 3-NPA toxicity than cortical region. These data reveal that alleviation bioenergetics will not be sufficient in the treatment of HD.

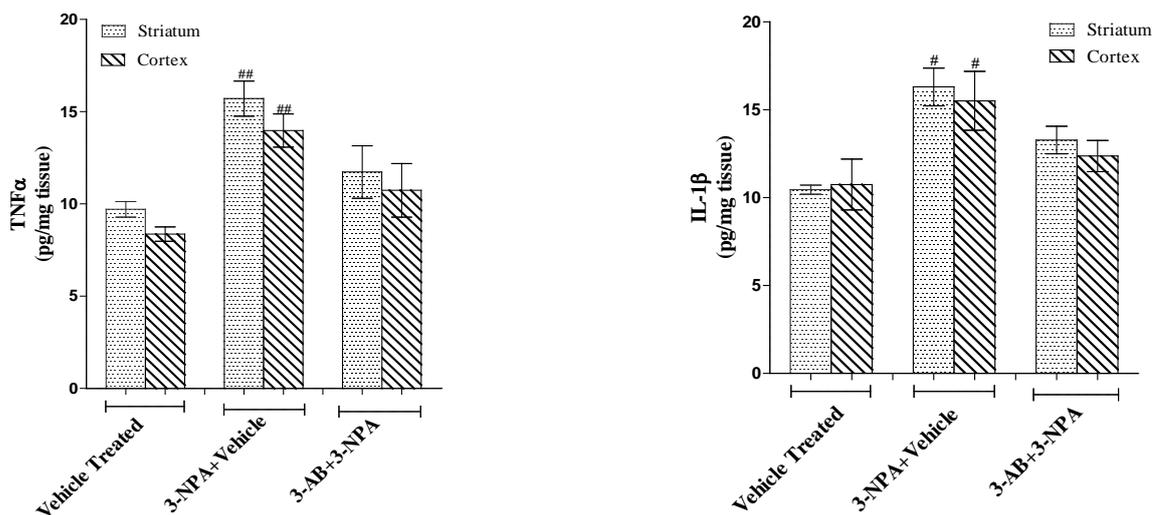


Fig. 6: Effect of 3-AB on inflammatory markers (TNF $\alpha$  and IL-1 $\beta$ ) in 3-NPA intoxicated mice brain. Values were expressed in mean $\pm$ SEM; n=3 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, #,## indicates p value<0.05 and 0.01, respectively Vs vehicle treated group

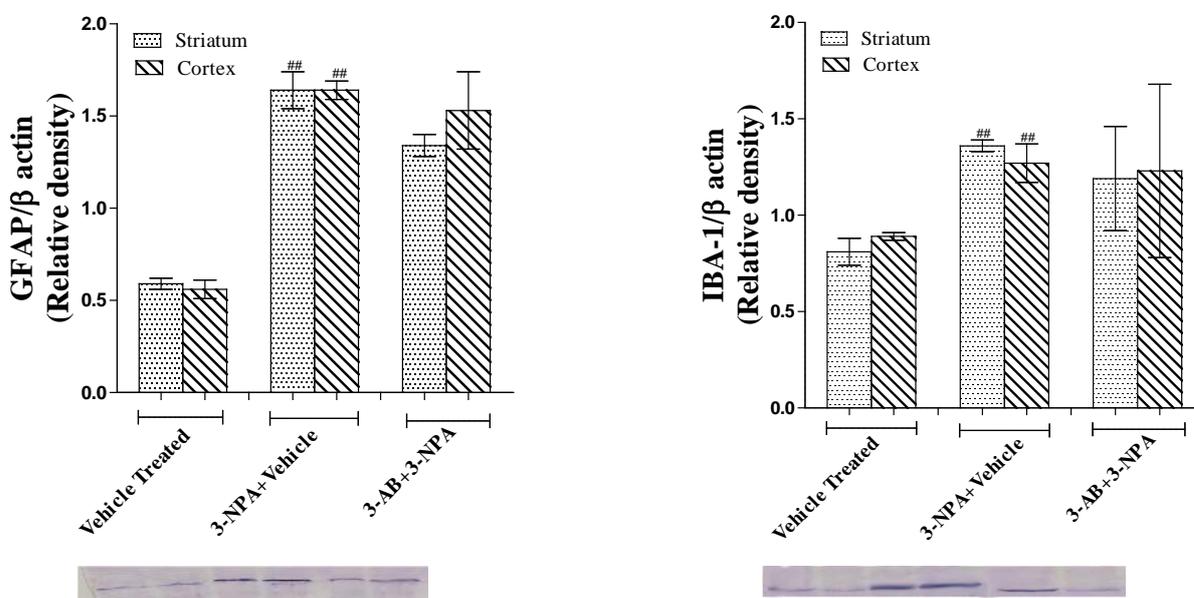


Fig. 7: Effect of 3-AB on glial markers (GFAP and IBA-1) in 3-NPA intoxicated mice brain. Values were expressed in mean $\pm$ SEM; n=4 animals/group; statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value<0.01 Vs vehicle treated group. Representative bands were shown below the graph. Lane 1 and 2 represents striatal and cortical vehicle treated mice brain; Lane 3 and 4 represents striatal and cortical 3-NPA intoxicated mice brain; Lane 5 and 6 represents striatal and cortical 3-AB administered mice brain

**CONCLUSION**

In conclusion, 3-AB plays a main role in restoring the succinate dehydrogenases activity and bioenergetics in 3-NPA mice brain. However, inhibition of glutamate excitotoxicity in turn gliosis and inflammation seem to be more essential in controlling the disease progression and secondary molecular events as well in HD than bioenergetics restoration.

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carry out the study. Authors also thank all technical and non-technical staff of CEFT for their help during study conduction.

**ABBREVIATION**

PARP1-Poly (ADP-ribose) polymerase 1; 3-AB-3-aminobenzamide; ATP-Adenosine triphosphate; NAD-Nicotinamide adenine dinucleotide; 3-NPA-3-nitropropionic acid; HD-Huntington's disease; TNF $\alpha$ -Tumor necrotic factor  $\alpha$ ; IL-1 $\beta$ -Interleukin 1 $\beta$ ; GFAP-Glial fibrillary acidic protein; IBA1-Ionized calcium-binding adapter molecule 1; CMC-Carboxy methyl cellulose; DCPIP-2,6-Dichlorophenol Indophenol.

**CONFLICT OF INTERESTS**

Author declares no conflict of interest

## REFERENCES

1. Cha JH. Transcriptional dysregulation in huntington's disease. *Trends Neurosci* 2000;23:387-92.
2. Rangone H, Humbert S, Saudou F. Huntington's disease: How does huntingtin, an antiapoptotic protein, become toxic? *Pathol Biol* 2004;52:338-42.
3. Estrada-Sanchez AM, Mejia-Toiber J, Massieu L. Excitotoxic neuronal death and the pathogenesis of Huntington's disease. *Arch Med Res* 2008;39:265-76.
4. Brouillet E, Jacquard C, Bizat N, Blum D. 3-Nitropropionic acid: A mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. *J Neurochem* 2005;95:1521-40.
5. Kumar P, Kumar A. Protective effect of rivastigmine against 3-nitropropionic acid-induced Huntington's disease like symptoms: Possible behavioural, biochemical and cellular alterations. *Eur J Pharmacol* 2009;615:91-101.
6. Túnez I, Santamaria A. Model of Huntington's disease induced with 3-nitropropionic acid. *Rev Neurol* 2009;48:430-4.
7. Oliver FJ, Menissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. *EMBO J* 1999;18:4446-54.
8. Chiarugi A, Moskowitz MA. Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. *J Neurochem* 2003;85:306-17.
9. Ullrich O, Diestel A, Eyupoglu IY, Nitsch R. Regulation of microglial expression of integrins by poly(ADP-ribose) polymerase-1. *Nat Cell Biol* 2001;3:1035-42.
10. Koh S, Chang D, Kim H, Kim J, Kim M, Kim KS, et al. Effect of 3-aminobenzamide, PARP inhibitor, on matrix metalloproteinase-9 level in plasma and brain of ischemic stroke model. *Toxicology* 2005;214:131-9.
11. Strosznajder R, Gajkowski B. Effect of 3-aminobenzamide on Bcl-2, Bax and AIF localization in hippocampal neurons altered by ischemia-reperfusion injury. The histochemical study. *Acta Neurobiol Exp* 2006;66:15-22.
12. Nam E, Lee YJ, Oh YA, Jung JA, Im HI, Koh SE, et al. Involvement of oxidative stress and Poly(ADP-ribose) polymerase activation in 3-nitropropionic acid-induced cytotoxicity in human neuroblastoma cells. *Korean J Physiol Pharmacol* 2003;7:325-31.
13. Babu CS, Ramanathan M. Pre-ischemic treatment with memantine reversed the neurochemical and behavioural parameters but not energy metabolites in middle cerebral artery occluded rats. *Pharmacol Biochem Behav* 2009;92:424-32.
14. Kalaivani P, Ganesh M, Gayathiri V, Jyothi Priya R, Pramila B, Sunil AG, et al. Alterations in SIRT1 and PARP1 expression precedes oxidative stress event in rats subjected to transient focal cerebral ischemia: Molecular and Histopathological evidences. *J Stroke Cerebrovascular Dis* 2014;23:2753-66.
15. Ludolph AC, He F, Spencer PS, Hammerstad J, Sabri M. 3-Nitropropionic acid-exogenous animal neurotoxin and possible human striatal toxin. *Can J Neurol Sci* 1991;18:492-8.
16. Tunes I, Tasset I, La Cruz VP, Santamaria A. 3-Nitropropionic acid as a tool to study the mechanisms involved in huntington's disease: past, Present and future. *Molecules* 2010;15:878-16.
17. Schallert T. Behavioural tests for preclinical intervention assessment. *Neurotherapeutics* 2006;3:497-504.
18. Kim ST, Son HJ, Choi JH, Ji IJ, Hwang O. Vertical grid test and modified horizontal grid test are sensitive methods for evaluating motor dysfunctions in the MPTP mouse model of Parkinson's disease. *Brain Res* 2010;1306:176-83.
19. Babu CS, Sunil AG, Hannah RV, Muthusamy VS, Ramanathan M. Development and validation of an HPTLC method for simultaneous estimation of excitatory neurotransmitters in rat brain. *J Liq Chromatogr Relat Technol* 2007;30:2891-902.
20. Bregman AA. *Laboratory Investigations In Cell Biology*, viii, Wiley, New York: Chichester; 1987. p. 268.
21. Zhan C, Yang J. Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Pharmacol Res* 2006;53:303-9.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
23. Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. *Physiol Rev* 2010;90:905-81.
24. Newmeyer DD, Ferguson-Miller S. Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* 2003;112:481-90.
25. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Sci* 2004;305:626-9.
26. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann Neurol* 1997;41:646-53.
27. Benchoua A, Trioulier Y, Zala D, Gaillard MC, Lefort N, Dufour N, et al. Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. *Mol Biol Cell* 2006;17:1652-63.
28. Wullner U, Young AB, Penney JB, Beal MF. 3-Nitropropionic acid toxicity in the striatum. *J Neurochem* 1994;63:1772-81.
29. Ranju V, Sathya S, Kalaivani P, Priya RJ, Babu CS. Memantine exerts functional recovery by improving BDNF and GDNF expression in 3-nitropropionic acid intoxicated mice. *Neurosci Lett* 2015;586:1-7.
30. Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt BR, Brundin P, et al. Increased sensitivity to N-methyl-D-aspartate receptor mediated excitotoxicity in a mouse model of Huntington's disease. *Neuron* 2002;33:849-60.
31. Schiefer J, Sprunken A, Puls C, Luesse HG, Milkereit E, et al. The metabotropic glutamate receptor 5 antagonist MPEP and the mGluR2 agonist LY379268 modify disease progression in a transgenic mouse model of Huntington's disease. *Brain Res* 2004;1019:246-54.
32. Lo EH, Bosque-Hamilton P, Meng W. Inhibition of Poly(ADP-Ribose) polymerase: reduction of ischemic injury and attenuation of N-Methyl-d-Aspartate-Induced neurotransmitter dysregulation. *Stroke* 1998;29:830-6.
33. Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, Li XJ. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *J Cell Biol* 2005;171:1001-12.
34. Jang M, Lee MJ, Kim CS, Cho I. Korean red ginseng extract attenuates 3-nitropropionic acid-induced huntington's-like symptoms. *Evidence-Based Complementary Altern Med* 2013;2013:1-17.