Original

Evaluation of biochemical and histopathological findings using Hesperetin against Huntington's disease like symptoms induced by 3-Nitropropionic acid in rats

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ABSTRACT

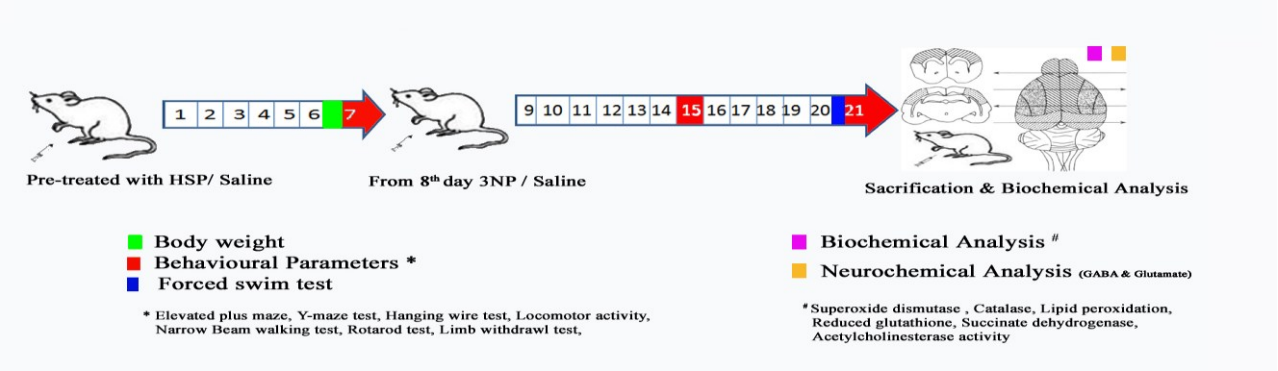
The disease Huntington's (HD) is an autosomal neurologic disorder characterized by inexorable loss of nerve cells in the brain accompanied with cognitive, motor and psychiatric disorders. In the present study, 3-Nitropropionic acid (3-NPA), an inhibitor of mitochondrial citric acid cycle results in symptoms like HD. Hesperetin(HSP) is a flavanone rich in citrus species which posses neuroprotective effects. The aim of the present study was to evaluate the protective role of HSP against 3-NP induced symptoms. Pre-treatment of animals with HSP/normal saline for 7 days and from 8th day, 3-NP (10mg/kg) was co-administered with HSP. It is continued for 21 days of the treatment schedule. At the end day of the study, the results showed that HSP improved all the cognitive, motor and psychiatric symptoms induced by 3-NP significantly. Further, oxidative stress parameters, enzyme succinate dehydrogenase and neurochemical estimations were done in the brain homogenate. The protective role of HSP further assessed by lesion area measurement with the 2,3,5-triphenyltetrazolium chloride staining of the brain slices and histopathological observations in hippocampus (CA1 and CA3) and striatum. Hence, these findings show the protective effect of HSP against 3-NP induced neurological disorder.

Keywords: *Huntington's disease, Hesperetin, 3-Nitropropionic acid, Oxidative stress, Histopathology*

1. INTRODUCTION

Huntington's disease is a chronic neurological disorder, characterized by inexorable loss of neurons in a group of contiguous subcortical structures in the region of basal ganglia. It affects the motor control and reward system in the brain (J. Leegwater-Kim et al., 2004). Striatum is the main region of basal ganglia facilitating the voluntary movements. This striatal cell loss leads to decline in cognitive, personality disorders and chorea. The pathogenesis of HD is mainly due to mitochondrial dysfunction (Wang LH et al., 2006). Normal condition of mitochondria is altered by the intereference of mutant Huntingtin protein (mHtt) causing inhibition of ATP production (Garcia M et al., 2002). Early findings showed that mitochondrial complex II enzyme Succinate dehydrogenase (SDH) of respiratory chain functioning is also related to the excessive levels of oxidative stress in the brain (I. Tunez et al., 2008). Excess production of reactive oxygen species is responsible for oxidative stress and cell damage which ultimately causes atrophy. In the brain naturally occurring reactive oxygen species is controlled by the cellular antioxidant systems (Wang M et al., 2006). If the balance between them is impaired it leads to mitochondrial dysfunction, oxidative stress and neuronal cell death. Previous studies have been supported that HD may be triggered by the mitochondrial dysfunctions (J.B. Schulz et al., 1996). Similar symptoms like HD can be induced by the chemical compounds that inhibit proper functioning of mitochondrial complex. Evidence states that there is no particular treatment for HD (M. Tariq et al., 2005). The cognitive and motor disturbances along with biochemical estimations were used to find the proper medication to this disease (Saydoff JA et al., 2003). Tetrabenazine was only approved drug by USFDA for the treatment of symptoms like chorea related to Huntington's disease, but found beneficial to some extent. However, different methods were used to treat HD but, unable to exhibit the particular reason to the cognitive impairment (S. Setter et al., 2009). So, there is a need to focus on the alternative methods to decrease the symptoms of the Huntington's like disease.Different models like injection of ibotenic acid, kainic acid, and quinolic acids in particular region of brain are exists. But

past years the well popular model 3-Nitropropionic acid (3-NP) model is used. 3-NP is a mitochondrial toxin produced by the fungal species and also from plant species (M. Tariq et al., 2005). It has the ability to cross the blood brain barrier; it acts as an irreversible mitochondrial SDH inhibitor. It shows mitochondrial dysfunction by decreasing ATP production which invariably leads to death of neurons (M. Ahuja et al., 2008). Reports have been



suggested that 3-NP acts primarily on striatal neurons causing atrophy. Moreover 3-NP also causes acute damage to the surrounding neuronal cells of the striatum such as cortex, hippocampus and hypothalamus (A.C. Ludolph et al., 1991).Flavonoids are rich antioxidants which comprise a group of phytonutrients seen in fruits and vegetables (Heim KE et al., 2002). They have great ability in inhibition of generative ROS and reduce the levels of formation of ROS (Giovanni Agati et al., 2012). Hesperetin (HSP; 3',5,7-Trihydroxy-4'-methoxy flavanone) a flavanone, from the group of flavonoids which is abundantly stored in the citrus species. It is aglycone of the flavonoid *Hesperedin (Garg A et al., 2001). It was reported that Neuroprotective effects of the citrus flavanones against H2O2-induced cytotoxicity in PC12 cells (Hwang SL et al., 2008). Based on the above factors, the present study aimed to evaluate the potentials of Hesperetin may improve the neuroprotection against 3-NP induced Huntington's disease in rats.*

Fig.1 Experimental Schedule

2. Materials and Methods

2.1. Chemicals

3-Nitropropionic acid (N5636-1 G, purity≥97%) , Hesperetin purchased from Sigma (USA), TTC (Triphenyl Tetrazolium Chloride) and all required chemicals were of analytical grade purchased from Hi-Media Laboratories Pvt, Ltd., Mumbai

2.2. Experimental Animals

Adult Wistar rats of either sex (220-250 gm) of Forty Eight in number, were purchased for this study. They were obtained from Mahaveer Enterprises, Hyderabad and the animals were housed with maintaining under standard laboratory conditions of light-dark cycle. Food and water were available *ad libitum*. Approval of protocol (ANUCPS/IAEC/AH/P/11/2018) was done by the Institutional Animal Ethics Committee (IAEC) of University College of Pharmaceutical Sciences, Acharya Nagarjuna University, according to the guidelines of CPCSEA, Govt. of India.

2.3. Experimental design

Forty Eight male Albino Wistar rats were grouped into four containing 12 animals in each group. The entire study was designed for 21 days as following schedule

Group 1: Saline (i.p)
Group 2: 3NP (10mg/kg, i.p)
Group 3: HSP (25mg/kg, p.o) + 3NP (10mg/kg, i.p)
Group 4: HSP (50mg/kg, p.o) + 3NP (10mg/kg, i.p)

From the 8th day of the treatment schedule 3-NP was administered by dissolving in normal saline intraperitoneally (i.p) with a dose of 10mg/kg/day. HSP was suspended in 0.1% carboxymethyl cellulose (CMC) and administered at a dosage of 25mg/kg/day and 50mg/kg/day, through Per Oral route (p.o) from day 1 to day 21 according to the treatment schedule. From the day 8 to day 21, HSP was administered 1 hour prior to the 3-NP treatment. After 3-4 hrs of treatment of animals different physical and behavioral parameters were analyzed on 7th, 15th and 21st day. Brain samples were collected by euthanizing animals using xylazine (10mg/kg, i.p) and ketamine hydrochloride (50mg/kg, i.p), for estimation of biochemical, measurement of brain lesion area (by TTC staining) and histopatological studies.

2.6. Preparation of brain homogenate

Six number of animals from each group were used for the estimation of biochemical and neurochemical parameters. The Brains of the animals were isolated by decapitation using deep anesthesia and are kept in ice-cold saline. Striatum, Cortex, Hippocampus was sectioned from the brain and homogenized in the ice-cold extraction buffer solution (10mM Tris HCL, pH 7.4, 10mM EDTA and 0.1% BSA). The homogenates were centrifugated at 4000×g at 4°C for 30min to obtain supernatants. The supernatants are re-centrifuged to collect mitochondrial pellets, washed with extraction buffer and the solution was again centrifuged at 4°C for 45min. The

pellets were re-suspended in buffer solution and were used for mitochondrial enzymes and some of the supernatants used for estimation of oxidative stress parameters and acetylcholinesterase before re-centrifugation after the mitochondrial estimations (R. Sandhir et al., 2010).

2.7. Assessment of Oxidative stress parameters

2.7.1. Superoxide dismutase

Enzyme Superoxide dismutase (SOD) modifies the superoxide radical into molecular oxygen (O_2) and Hydrogen peroxide (H_2O_2). Eventually using the enzyme catalase H_2O_2 will convert into H_2O andO_2. So, the main enzymatic function is to convert the harmful molecules into water and oxygen. According to Markund et al., the ability of the enzyme is based on the inhibition of pyrogallol autoxidation. Reaction mixture of about 3 ml was prepared by 0.5ml pyrogallol (0.2mM) with HCl , 2ml of Tris HCl buffer and 0.5ml of distilled water .this mixture was subjected to change in the absorbance at 420nm with 1 min time interval. Homogenate mixture was added instead of distillated water to the reaction mixture and the same procedure is followed for change in the absorbance at 420nm. The inhibition percentage of pyrogallol autoxidation is measured after homogenate addition. 50% of inhibition was considered as the one unit of required enzyme (S. Marklund et al., 1974) .

% Inhibition = [Control dA/dt- Sample dA/dt/ Control dA/dt] ×100

2.7.2. Catalase

Enzyme Catalase is responsible for the conversion of H_2O_2 into water (H_2O) and oxygen (O_2), thereby reducing the oxidative damage in tissues. Based on the method of Claiborne (1985) with small changes catalase was assayed. In this method, 1.9ml of Phosphate buffer (0.05M), 30 mM H_2O_2 of 1 ml and the reaction was started by the addition of 0.1 ml of the homogenate. A gradual decrease in the absorbance by conversion of H_2O_2 was measured with a 1min interval at 240nm. Calculation of catalase activity with an extinction coefficient of 43.6 M^{-11} cm^{-1} and units as per g wet weight of tissue (A. Claiborne et al., 1985).

2.7.3. Lipid peroxidation

Malonaldehyde (MDA) produced by cell injury was used to measure the lipid peroxidation. In the present study, MDA levels present in the tissue homogenate were used to measure the cell injury. According to ohkawa, et al. to the 0.1ml of tissue sample, 2ml of thiobarbituric acid reaction mixture containing trichloroacetic acid (1ml of 10% solution) and thiobarbituric acid (1ml of 0.67% solution). Then the reaction mixture was boiled for about 30 min in a water bath and it was cool for 10 min in an ice bath. The supernatant was collected by centrifuging the reaction mixture after cooling about 10 min at 5000×g and absorbance at 532 nm was observed. An extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used for calculation and values expressed as nmol MDA/g wet weight of tissue (D. Gelvan et al., 1990).

2.7.4. Reduced glutathione

Reduced glutathione is assessed according to the Ellman et,al. with minor changes. Similar volumes of the sample tissue homogenate and trichloroacetic acid (10%) were mixed and centrifuged about 20min, 1 ml of supernatant was separated and added 3ml of phosphate buffer (0.2M, pH 8) ,0.5ml of DTNB reagent (0.6mM in 0.2M phosphate buffer). Absorbance was measured after mixing the solutions. An extinction coefficient of 1.36×10^3 M^{-1} cm^{-1} was used for calculation and expressed values as nmol GSH/g wet weight of tissue (Kumar P et al., 2009).

2.7.5. Succinate dehydrogenase (SDH)

The conversion of the succinic acid into fumaric acid in the presence of potassium ferricyanide is the main principle involved in the estimation of SDH. The reaction mixture phosphate buffer of 1.5 ml , 0.1ml of potassium ferricyanide (0.3M), 0.2ml of succinic acid(0.6M, pH7.8) , 0.3ml (1% w/v) of BSA and mitochondrial suspension of 0.05ml was added. For assessing the succinate dehydrogenase, absorbance at 420nm shows decrease while using water as a blank for 3 min. The expression of values is nmol succinate oxidized / min /g wet weight of tissue (T.E. King et al., 1967).

2.7.6. Acetylcholinesterase activity

Acetylcholine in the tissues was measured indirectly by the acetylcholinesterase(AChE) activity. Levels of acetylcholine decrease due to hydroxylation by acetylcholinesterase. 2.6 ml of Phosphate buffer (0.2 M, pH 8) and 100 µl of DTNB and 0.4ml of homogenate were mixed well and absorbance at 412 nm is measured. Change of absorbance recorded for 1 min by the addition of acetylthiocholine of 20 µl and absorption noted for 5 min with 1 min time interval. Estimation of AChE done by using equation $R=5.74\times10^{-4}$ × absorbance change per min /g wet weight of the tissue (G.L. Ellman et al., 1961).

2.8. Neurochemical analysis

Assessment of Glutamate and GABA was carried out according to the Maynert et al. with very minor changes. The hemispheres of half the brain was sectioned in to striatum, hippocampus and cortex and washed with 80% ice-cold ethanol. To prepare 10% homogenates of three regions they were centrifuged with 80% ice-cold ethanol. These homogenates individually centrifuged at 9000×g for separation of supernatants and sediments. Sediments separated were used for extraction of Glutamate and GABA by repetition of extract with 80% of the ice-cold ethanol. Above 3ml of extract used for evaporation of ethanol from all three samples, residue was constituted with distilled water of 100 ml. Glutamate and GABA of standard concentrations (2mM) were prepared and spotted using paper chromatography. Chromatography chamber with a composition of butanol :aceticacid :water (12:3:5 v/v) was used for placing the spotted chromatography papers and were dried repeatedly. Ninhydrin reagent was

sprayed on dried papers and placed in an hot airoven for about 4 min at the temperature of 100° C. The spots sampled were identified similar to the standards (GABA and Glutamate) and spotted areas were cut to be used for eluting the contents with 0.005% $CuSO_4$ in 75%ethanol. The elutes were observed at absorbance of 515nm using spectrophotometer (P.K. Kola et al., 2017).

2.9. Brain lesion area

The brains of the three animals of each group were isolated using deep anesthesia and kept at -4° C for 20 minutes. They were coronally sliced from frontal pole with thickness of 2 mm by razor blade. 2,3,5 triphenyltetrazolium chloride (TTC) was an immersion method used for the measurement of brain infract area. The coronal sections were placed in a petridish consisting of 2,3,5 TTC in normal saline about 2% and they were covered with glass slips for having even spread of TTC solution. The petridishes were closed with aluminum foil and incubated at 37° C for 30minutes by preventing light exposure. Then the TTC again replaced with 10% neutral-buffered formalin and allowed to settle over-night. The high resolution images of the sections were taken and measurement of brain lesion area was done using the Image. J. 1.50 software (INH, USA). It was noted as percentage (%) of lesion area of the total brain area (J.B. Bederson et al., 1986).

2.10. Histopathology

Three animals of each group were anesthetized and the brain samples were isolated and stored in formalin (10% v/v) on the day 22nd for the histopathological studies. The samples were dehydrated with different volumes of alcohol and they were cleaned with xylene after 24 hours. Later waxing was done for coronal sectioning of the brain sample into 5µm thick slices with the help of an apparatus, Microtome (Medimeas Ins-MRM1120). Hematoxylin and eosin stains were used for the staining of slices to observe the viability of neuronal cells in the striatum and hippocampus (CA1 and CA3). The neuronal cell count was examined by the light microscope under 400X, with the help of the software Image J. they were quantified. Values expressed as mean of the coronal sections of animals.

2.11. Statistical findings

The values are noted as mean ± SEM. The neuronal data score was assessed by using kruskal wallis analysis of variance (nonparametric), and all parameters were assessed by one way ANOVA followed by the method named, tukey's test using the software Graph pad prism. Statistical significance was set at $P \leq 0.05$.

3.3. Biochemical estimations

3.3.1. Oxidative stress parameters

The normal levels of SOD, CAT, GSH were reduced and the levels of MDA was increased due to the administration of 3-NP compared to the normal group in the regions of striatum, cortex and hippocampus. The low dose and high dose of HSP treated groups significantly($P < 0.05$) increased the amounts of SOD, CAT, GSH and MDA levels were decreased when compared to 3-NP lone treated group in the areas of striatum, cortex, hippocampus.

Table. 2

Effect of HSP on oxidative stress parameters in the cortex of 3NP toxicity study

Treatment	MDA nmol/gm wet weight tissue	GSH µmol/gm wet weight tissue	CAT µmol/min/gm wet weight tissue	SOD Units/gm wet weight tissue
Normal	50.6±2.64	2.16± 0.185	26.7±2.05	28.3±1.48
3NP	155±16.7***	0.622±0.126***	8.99±0.888***	9.89±2.35***
HSP(25mg)	97.4±4.85$^{\#\#}$	1.40 ± 0.0533$^{\#}$	17.8±1.33$^{\#\#}$	19.1±1.93$^{\#}$
HSP(50mg)	63.1±5.53$^{\#\#\#}$	1.59± 0.136$^{\#\#}$	22.5±0.955$^{\#\#\#}$	22.9±0.883$^{\#\#}$

Results are expressed as mean ± SEM (n=6); *** indicate $P < 0.001$, respectively, Vs control; $^{\#,\#\#}$ and $^{\#\#\#}$ indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, Vs 3-NP.

Table. 3

Effect of HSP on oxidative stress parameters in the Striatum of 3NP toxicity study

Treatment	MDA nmol/gm wet weight tissue	GSH µmol/gm wet weight tissue	CAT µmol/min/gm wet weight tissue	SOD Units/gm wet weight tissue
Normal	52.05±2.074	2.266±0.190	27.32±1.486	27.89±1.121
3NP	155.7±11.12***	0.6400±0.150***	8.865±0.856***	10.72±2.192***
HSP(25mg)	97.92±2.907$^{\#\#\#}$	1.553±0.052$^{\#\#}$	18.54±1.288$^{\#\#}$	18.37±1.903$^{\#}$
HSP(50mg)	69.12±2.111$^{\#\#\#}$	1.939±0.081$^{\#\#\#}$	22.44±0.673$^{\#\#\#}$	24.11±0.943$^{\#\#}$

Results are expressed as mean ± SEM (n=6); *** indicate $P < 0.001$, respectively, Vs control; $^{\#,\#\#}$ and $^{\#\#\#}$ indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, Vs 3-NP.

Table. 4

Effect of HSP on oxidative stress parameters in the hippocampus of 3NP toxicity study

Treatment	MDA nmol/gm wet weight tissue	GSH µmol/gm wet weight tissue	CAT µmol/min/gm wet weight tissue	SOD Units/gm wet weight tissue
Normal	39.2 ±4.32	2.59±0.220	28.5±1.55	25.5±1.83
3NP	164 ±6.24***	0.845±0.257**	12.1±2.11***	7.88±1.10***
HSP(25mg)	104 ±8.61##	1.50±0.193#	19.7±0.313#	14.0±1.13#
HSP(50mg)	74.5 ±6.90###	2.24±0.163##	22.8±0.871##	19.8±0.820###

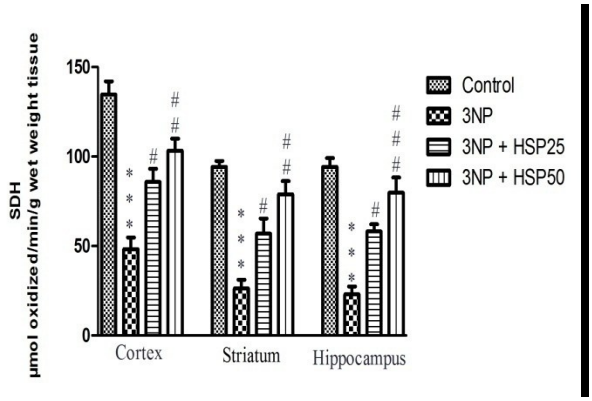
Results are expressed as mean ± SEM (n=6); *** indicate P < 0.001, respectively, Vs control; #,## and ### indicate P < 0.05, P < 0.01, and P <0.001, respectively, Vs 3-NP.

3.3.2. Succinate dehydrogenase

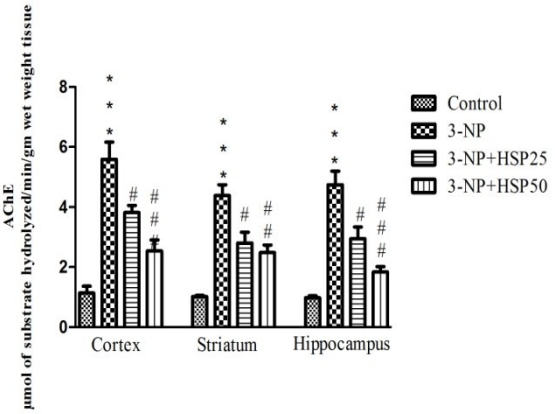
Administration of 3-NP decreases the SDH activity in the cortex, striatum and hippocampus regions of the brain significantly when compared with normal control animals. HSP treated both groups (25mg/kg and 50mg/kg) enhanced the level of SDH of all three regions of brain when compared with of 3-NP alone treated group, P < 0.05 is the significant value of the activity.

3.3.3. Acetylcholinesterase activity

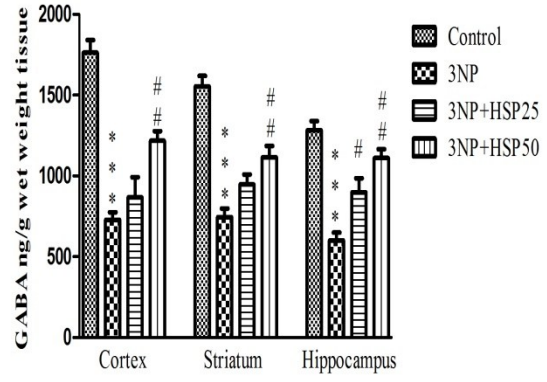
By the treatment of a mitochondrial toxin, 3-NP significantly increases the levels of AChE in the cortex, striatum, hippocampus of the brain region when compared with normal control animals. Animals treated with low and high doses of HSP was significantly reduced (P < 0.05) the levels of AChE when compared with the 3-NP alone treated group.



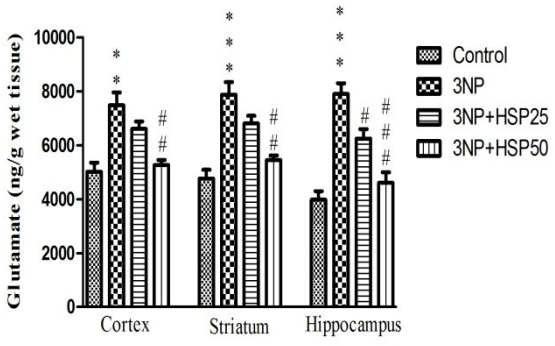
(a) SDH levels



(b)AChE



(c) GABA



(d) Glutamate

Fig. 5 Effect of HSP on (a) SDH levels, (b) AChE levels, (c) GABA and (d) Glutamate. Results are expressed as mean ± SEM (n=6); *** indicate P < 0.001, respectively, Vs control; #,## and ### indicate P < 0.05, P < 0.01, and P <0.001, respectively, Vs 3-NP.

3.4. Neurochemical analysis

Neurotransmitters were significantly altered after administration of 3-NP by reducing the GABA levels and enhancing the glutamate levels when compared with normal group of animals. The treatment groups of both low dose and high dose of HSP alters significantly (P < 0.05) by increasing levels of GABA and lowering the levels of glutamate dose dependently at all three region of the brain.

3.5. Brain lesion area

3NP administered group showed the lesion of 49.2% in the total area of the brain. The low and high doses of HSP treated groups showed significantly reduced the lesion area of brain by 28.36% ($P < 0.01$) and 19.64% ($P < 0.001$) respectively when analyzed with 3-NP alone treated group.

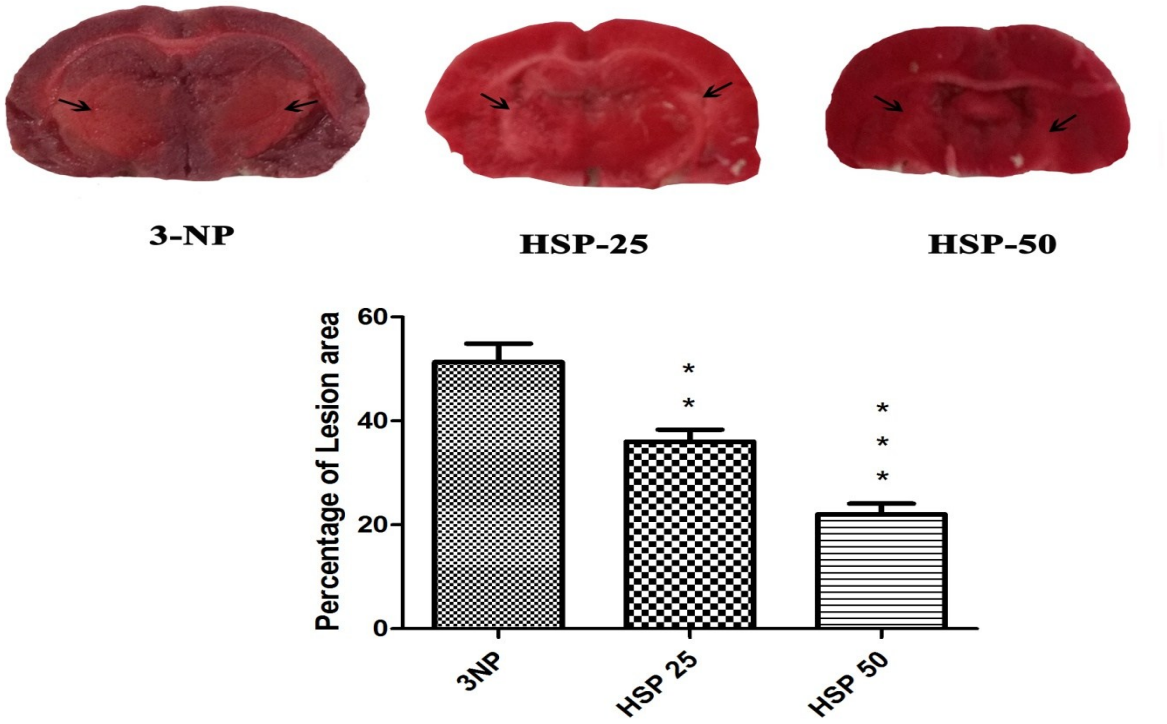
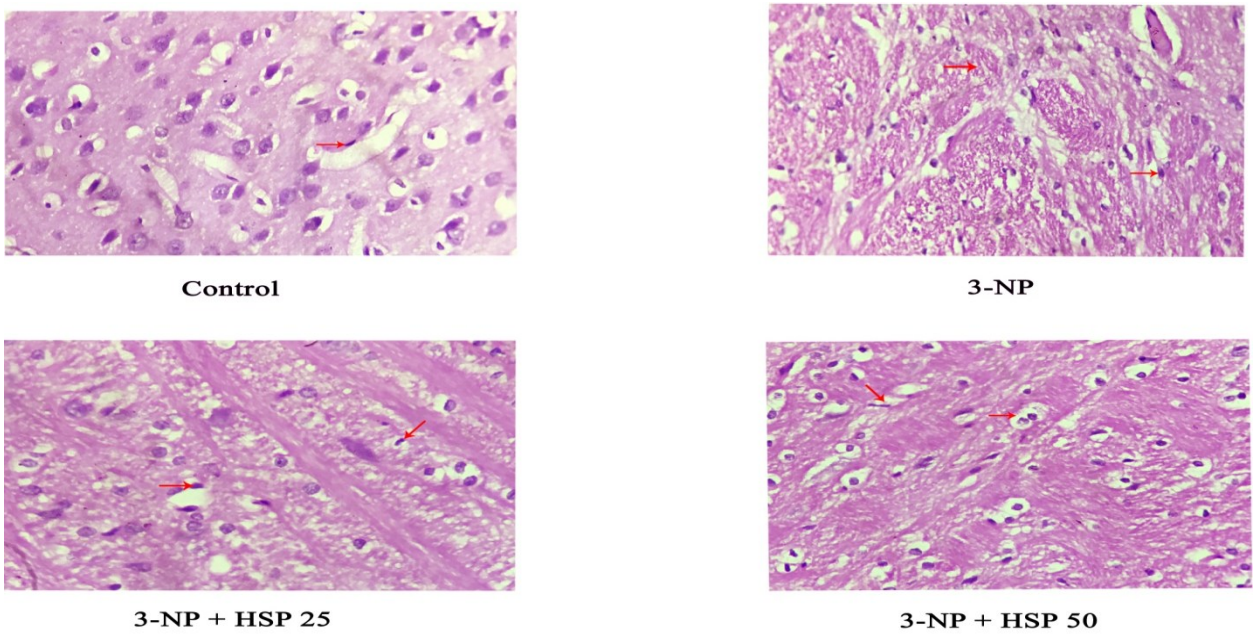


Fig. 6 Effect of HSP on brain lesion are in the 3-NP induced neurotoxic rats. The values expressed as mean ± SEM (n=3); **, *** indicate $P < 0.01$, $P < 0.001$, respectively Vs 3NP.

3.6. Histopathology

By examining histopathology, structural modifications in the striatum and the hippocampus were observed. Normal histological structures were observed in normal control group. Distinct fibrillar inclusions with hematoxylin and eosin stain were observed in 3-NP alone treated group indicating neurofibrillary degeneration with edema in perivascular area and shrinkage of cells in the striatum region. In the HSP treated group, improved histological changes were observed in the striatal region of the brain. The CA1 and CA3 areas in the hippocampus of brain were assessed for viable neuronal cell count. The significant decrease in the viable cell count was observed in 3-NP alone group when compared to normal control animals. HSP treated group showed remarkable increase in viable cell number in CA1 and CA3 hippocampal regions of brain.



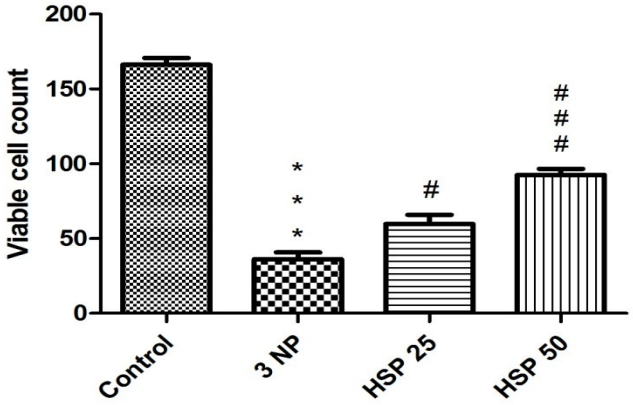
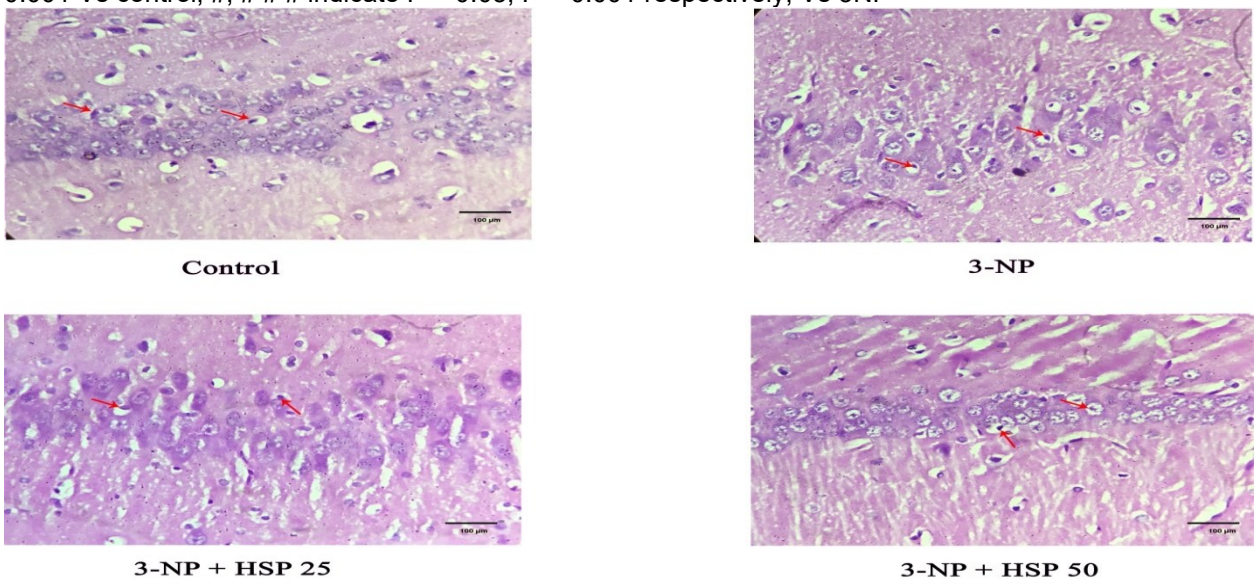


Fig. 7 Effect of HSP on 3NP induced histological modifications (400X) in the region of striatum region of the brain (pink arrows indicates fibrillary damage and black arrow indicates perinuclear and perivascular edema). The graph indicates the viable cell count and the values are represented as mean ± SEM (n=3); *** indicate P < 0.001 Vs control; #, # # # indicate P < 0.05, P < 0.001 respectively, Vs 3NP



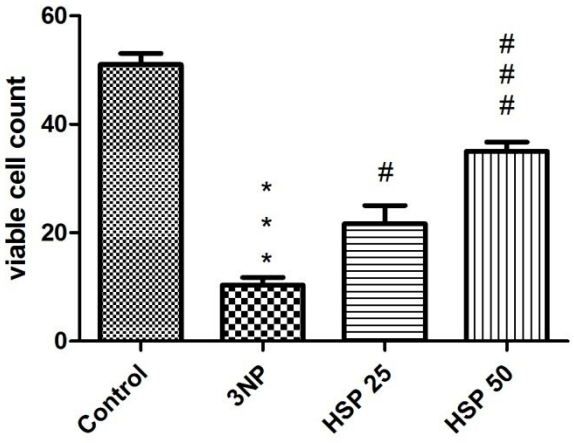


Fig. 8 Effect of HSP on 3NP induced histological modifications (400X) in the region of hippocampus, CA1 regions of the brain (arrows indicates the pyknotic cells). The graph indicates the viable cell count and the values are represented as mean ± SEM (n=3); *** indicate P < 0.001 Vs control; #, # # # indicate P < 0.05, P < 0.001 respectively, Vs 3NP

Cognitive impairment in the brain is related with the hippocampal CA1 and CA3 pyramidal neuronal degeneration by the induction of 3NP (P. Kumar et al., 2009). In the present study, the 3NP administered group showed significant memory impairment in the elevated plus maze test and Y maze test. Treatment with HSP

significantly improved the cognitive impairment based on the performance of the both tests; the findings suggest the ability of HSP against 3NP induced neuronal damage in HD.

Based on the HD affected patients, neuronal damage and increased oxidative stress, enhance the neurotransmitters of various regions of the brain which forms the anxiety and depression (D. Craufurd et al., 2001). 3-NP causes neuronal damage in the basal ganglia and the depression like symptom (D. Jain et al., 2014). In the present study, by administering the 3-NP in rats showed a significant increase in immobility in forced swimming test, indicating the depression behavior of the animal. The HSP administered animals restores antioxidant mechanism in different areas of brain which may be a promoting factor of antidepressant activity.

Oxidative stress and the mitochondrial dysfunction are also responsible for the Pathophysiology of Huntington's disease. Reported data show that 3-NP intoxication causes the oxidative stress by inhibition of the mitochondrial enzyme, SDH and impairment in metabolization of energy (R.F. Villarán et al., 2008). Increased levels of lipid peroxidation in the brain tissue, thereby increased levels of MDA and the decreased levels of antioxidant defense mechanism like GSH, SOD and CAT are also occurring due to the administration of 3-NP(P. Kumar et al., 2010). Similarly to the give data, in this present study the 3-NP administered animals shows the significant decreased levels of GSH, SOD, CAT and increase in the MDA levels significantly in the brain regions. The HSP treated animals restored the antioxidant defense mechanism significantly and inhibit lipid peroxidation by decreasing the MDA levels in the brain regions such as cortex, striatum and hippocampus. Some of the studies indicate that neuronal toxicity induced by the 3-NP ameliorated by the antioxidants like lycopene and sesamol (B.D. Shivasharan et al., 2013). Most of the previous studies state the effective role of antioxidant plays a crucial role in ameliorating the toxic effects of 3-NP. In this present study, HSP decreases the unbalanced levels of lipid peroxidation, shows that it may be due to its antioxidant properties by improving neuroprotection against 3-NP toxicity.

In Huntington's patients, an unswerving defect in II and IV of mitochondrial complex in the striatum area of the brain have identified (Sumathi Thangarajan et al., 2016) and demonstration of R6/2 HD model states that the mHtt interfere with energy metabolism by transcription of receptor gamma coactivator-1α (PGC-1α) due to repression of peroxisome (L. Cui et al., 2006). In experimental procedure, 3NP binds irreversibly with the complex II (SDH), which inhibits the free fatty acid oxidation resulting impairment in energy metabolism. The interruption in mitochondrial enzymes is responsible for the generation of massive ROS (Reactive Oxygen Species) (A. Hariharan et al., 2014). Thus, mitochondrial function in the brain can be improved by amelioration of the 3NP toxicity. The values obtained are similar to the previous studies, showing the neurotoxicity effects of 3-NP significantly, by decreasing the SDH activity in the striatum, cortex and hippocampus. The pretreatment of HSP before 3-NP administration restored the SDH activity by depending on dose. Studies also explained that neurotoxicity will be mitigated either by overexpression of the enzyme, SDH of the mitochondria or by upregulation of mitochondrial biogenesis (L.W. Chen et al., 2012). Recent studies exhibited that the inhibition of SDH in 3-NP toxicity was prevented by the pretreatment of medicinal agents (R. Sandhir et al., 2013). Hence protective effect of HSP suggests its ability to regain the SDH activity in the striatum, cortex and hippocampus in brain against 3-NP neuronal toxicity.

The acetylcholine in the brain are responsible for the cognitive functions, the decline in the cognitive performances in HD patients, showed the increased levels of acetylcholine in the brain (B.V. Manyam et al., 1990). The levels of acetylcholinesterase activity have to be reduced in the brain. In the present study similar to this, 3-NP administration causes the increased cholinesterase activity in the cortex, striatum and the Hippocampus of the brain. By the treating with HSP significantly reduced the acetylcholinesterase activity dose- dependently at all the three regions of the brain. Hence, the HSP enhances the cognitive functioning by reducing the acetylcholinesterase activity in the brain.

The neurochemical alteration is also responsible for the pathophysiology of HD patients and as in the animal experimental models. Based on the earlier studies, it is known that administration of 3-NP enhanced the glutamate levels and reduced the GABA levels in the brain (L. Wang et al., 2017). As expected, the administration of 3NP improved the levels of glutamate and decrease the GABA in the three regions of the brain. The treatment of HSP attenuates the excitotoxicity by a reduction in the levels of glutamate and by elevating GABA levels in the brain. Hence these results show a link between neurochemical and behavioural changes, suggests that HSP has a capacity to attenuate the HD like symptoms caused by 3-NP.

TTC staining method is used to measure the affected area of the brain, in support to the neuroprotective potential of HSP against 3-NP toxicity. In previous studies, the bilateral striatal lesions in the brain was observed due to administration of 3-NP. The HSP treated animals showed the reduced lesion area dose -dependently indicates the protective effect against 3-NP induced neuronal damage. HSP was later assessed by histopathology of striatum and hippocampus (CA1 and CA3) regions. According to the previous study, the administration of 3-NP causes the pyramidal neuronal cell loss in the regions of CA1 and CA3 regions of hippocampus (T. Sugino 1999). In the present study, HSP significantly decrease in the pyramidal neural cell count in regions of the CA1 and CA3 of hippocampus in the brain.

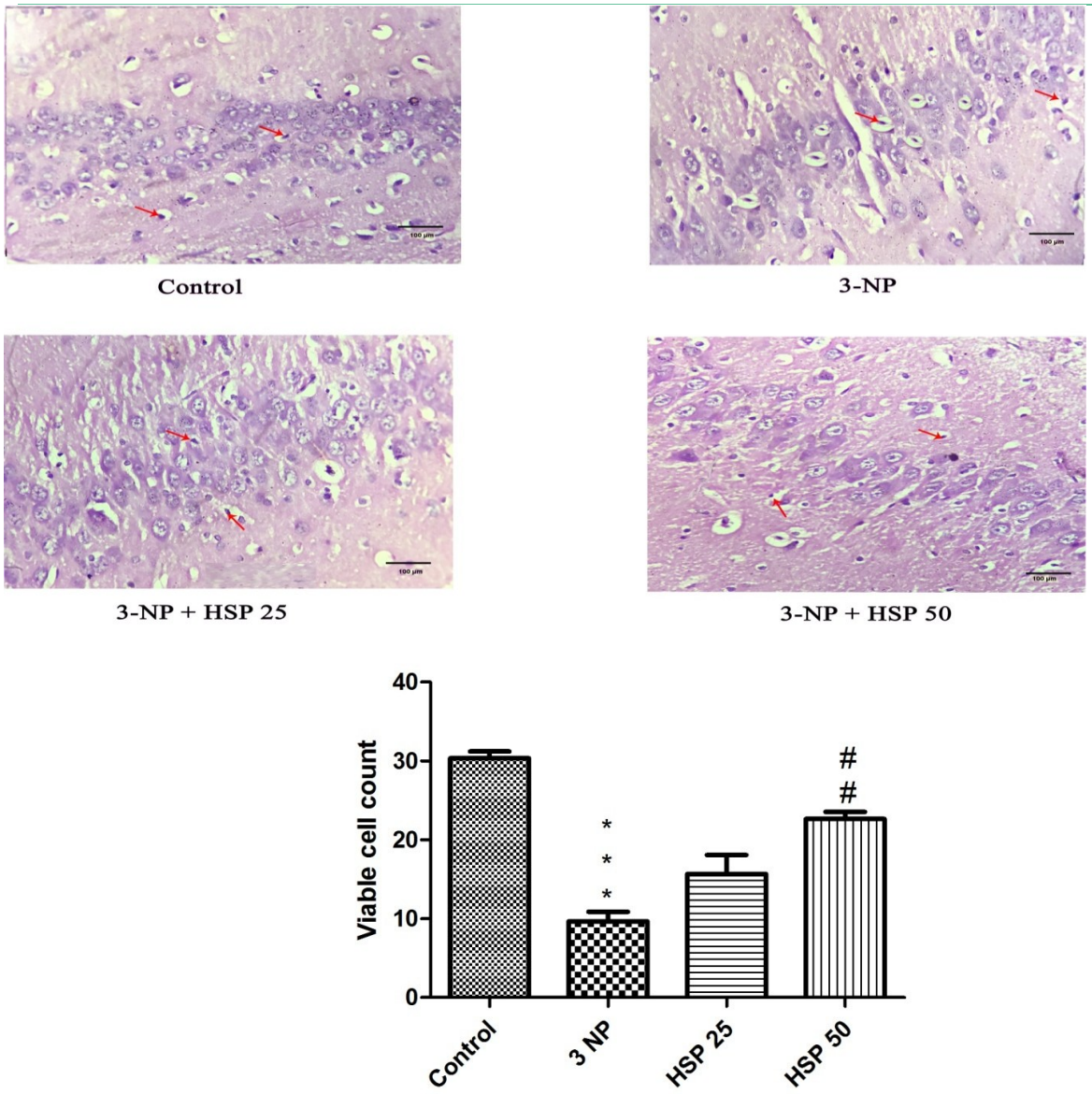


Fig. 9 Effect of HSP on 3NP induced histological modifications (400 X) in the region of hippocampus, CA 3 regions of the brain (arrows indicates the pyknotic cells). The graph indicates the viable cell count and the values are represented as mean ± SEM (n=3); *** indicate P < 0.001 Vs control; ## indicate P < 0.01 respectively, Vs 3NP

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