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Immunohistochemical Studies on HDAC-4 in Experimentally Induced Parkinson's Diseases

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Abstract

The environmental toxin hypothesis was dominant for much of the 20th century, and posits that PD-related neurodegeneration results from exposure to a dopaminergic neurotoxin. Living in a rural environment appears to confer an increased risk of PD, perhaps due to increased exposureto pesticide use and wood preservatives. Cigarette smoking and coffee drinking are inversely associated with the risk for the development of PD. These modifications are likely to contribute to the onset and progression of complex human diseases including neurodegenerative ones. Oxidative stress also is thought to be a common underlying mechanism that leads to cellular dysfunction and demise in PD. This study was aimed to assess the effect of butyric acid in PD experimental model through HDAC activities. The study was carried out on five rat groups, control group, Parkinsonism group, and sodium butyrate group, two Parkinson's disease groups co-administered and post treated with sodium butyrate. Parkinsonism was induced by ip injection of paraquat. Laboratory measurements included serum HDAC activity and HDAC-4 antibody stain. PD group, PD co-administered and post treated with sodium butyrate showed significant increase in HDAC activity. Histological and Immunohistochemical investigate. The increments in HDAC activities are one of the pathogenic mechanisms of the disease or it affords PD patients neuroprotection and benefits. Also, sodium butyrate is one of best antioxidant and neuroprotective agents. We recommended for further studies in HDAC and sodium butyrate as inhibitor in neurodegerative diseases, other diseases and normal state. Further investigation is required by determining activity of HDAC to clarify its role in Parkinsonism.

Keywords

Parkinson's Disease, Intra Peritoneal, Histone Deacetylase, Methy Phenyl Tetrahydro Pyridine, Histone Acetyl Transferase, Sodium Butyrate

1. Introduction

Until now, very little is known about why and how the PD neurodegenerative process begins and progresses. Yet over the last two decades, tremendous strides toward acquiring a better knowledge of both the etiology and pathogenesis of PD have been achieved, thanks to numerous elegant clinical studies and investigations performed in autopsy materials and in vitro and in vivo experimental models of PD (Pan-Montojo and Reichmann, 2014).

Despite these unquestionable advances, there is a major gap in understanding the molecular and cellular biology of PD. Consequently, investigators still rely heavily on experimental models of PD to obtain greater insights into its cause, but more particularly into its pathogenesis. Moreover, Because PD appears to be a multifactorial disorder, the use of animal models to investigate additive or synergistic effects of environmental risk factors are of great importance, especially in the context of aging, which is the single major risk factor for the disease (Peng et al, 2007).

Among the various accepted experimental models of PD, neurotoxins have remained the most popular tools to produce selective neuronal death in both in vitro and in vivo systems

(Hisahara and Shimohama, 2010). The key neurotoxic models of PD, namely those produced by the toxins 6-hydroxydopamine (6-OHDA), MPTP, rotenone, and paraquat (PQ) (Hisahara and Shimohama, 2010).

Paraquat (PQ; 1, 1-dimethyl-4, 4-bipyridinium dichloride) is a highly toxic quarternary nitrogen herbicide. Because of its low cost, rapid action, and environmental characteristics, paraquat is a widely used herbicide around the world (Franco, et al., 2010). This widely used herbicide is considered a prime risk factor for PD based on both epidemiological evidence of increased incidence of PD after exposure and its chemical similarity to the parkinsonism-inducing agent MPTP (Dinis-Oliveira, et al., 2008) (figure 1).

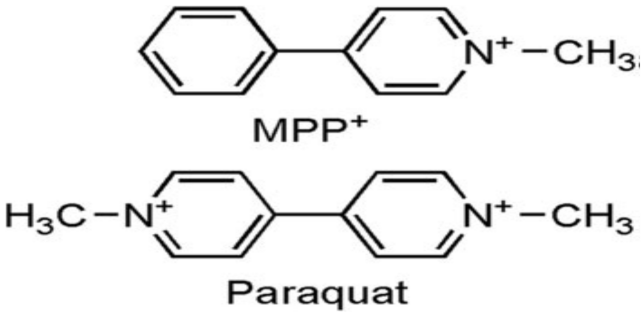

Fig. 1. *Chemical structures of MPP+ and paraquat (Hisahara and Shimohama,2010).*

For many years, experimental studies using paraquat were focusing on its effects on lung, liver, and kidney probably because the toxicity induced by this herbicide in these organs is responsible for death after acute exposure. However, significant damage to the brain is seen in individuals who died from paraquat intoxication (Nisticò et al., 2011), despite the fact that paraquat poorly crosses the BBB spontaneously. paraquat did enter the brain via the assistance of L-neutral amino acid transports, as pretreatment of animals with L-valine or L-phenylalanine completely prevented neurodegeneration (Cannon and Greenamyre, 2011).

Chronic paraquat exposure has been suggested as an etiological factor for PD. Animal studies have demonstrated that paraquat can cause dopaminergic neuronal degeneration in both cell culture and animal models, induce Parkinsonian-like symptoms in animals, and promote α-synuclein-positive cellular inclusions similar to Lewy bodies (Kanthasamy et al., 2008). In addition, the study of paraquat's neurotoxic properties has provided valuable information regarding the potential mechanisms involved in the progression of neurodegeneration associated with environmental toxicity (Franco et al., 2010).

Protein lysine acetylation refers to post-translational addition of an acetyl moiety to the ε-amino group of a lysine residue (Figure 2). This reversible modification, also known as N-acetylation, was first discovered in the early 1960s, with the chemical nature defined as Ne-acetylation a few years later (Allfrey et al., 1964).

A decade ago, HATs were first reported to acetylate the tumor suppressor p53 and two general transcription factors leading to the notion that HATs and HDACs are not just for histones (Ropero and Esteller, 2007). As a result, many investigators rushed to test various transcription factors. Since then, over 60 of them have been shown to be subjected to N ε-acetylation (Gre´goire et al., 2007). Importantly, this has been extended to many regulators of DNA repair, recombination and replication; viral proteins; classical metabolic enzymes, such as bacterial and mammalian acetyl-CoA synthases; and recently to kinases, phosphatases and other signaling regulators. Moreover, three proteomic studies identified acetyl lysine residues in a diverse array of proteins (Xie et al., 2007).

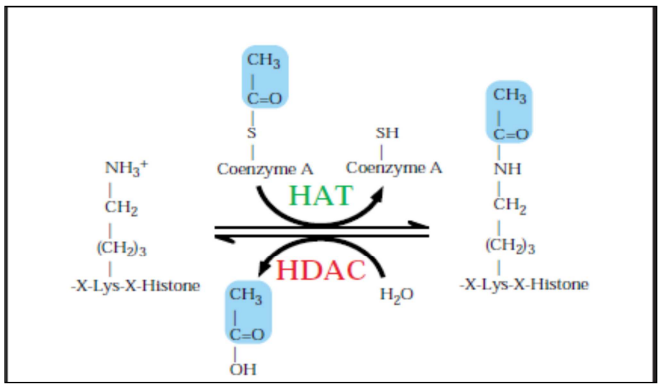

Fig. 2. *Equilibrium of steady state histone acetylation is maintained by opposing activities of HAT and HDAC (Allfrey et al., 1964).*

Given that HDACs are involved in regulation of non-histone proteins and also act at the chromosome level to regulate gene transcription, it is not surprising that these multi-complex enzymes are involved in various cellular processes such as differentiation (Singh et al., 2010), DNA replication (Telles and Seto, 2012) and cell cycle progression (Yamaguchi, et al., 2010). A large number of HDACs have been demonstrated to have important functions in neurons. Much information gained from the use of pharmacological HDAC inhibitors is available (Morrison et al., 2007).

Sodium butyrate could provide energy for normal colonic mucosal epithelial cells and maintain their renewal (Blottiere et al., 2003). Camarero et al. (2003) believe that sodium butyrate inhibits cell growth through inducing the expression of mitochondrial HMG-CoA synthetase, leading to accumulation of mid-products of acetyl-CoA and β-oxidation, and inhibition of acetyl-CoA dehydrogenase.

To date, the most notable effect of butyrate that has been observed on cultured cells has been its ability to induce hyperacetylation of the histone proteins; with histones H3 and H4 being most affected (Vidali et al., 1978). This effect is reversible and is a direct consequence of the inhibition of the deacetylase enzyme(s) (Cousens et al., 1979). Butyrate has also been shown to prevent phosphorylation of histones H1 and H2A (Boffa et al., 1981), apparently mediated through the inhibition of certain kinases. Hyperphosphorylation of the nuclear proteins HMG 14 and 17 caused by butyrate treatment has also been reported (Levy-Wilson, 1981). Methylation of certain cellular proteins is inhibited by this fatty acid, probably due to the inhibition

of some methylase enzymes and not due to a reduction in the pool of S-adenosyl-L-methionine (Boffa et al., 1981). Sodium butyrate has been shown to enhance α-fetoprotein, albumin, and transferrin production in a rat hepatoma cell line (Schut et al., 1981) and to cause neurite-like process formation in a mouse neuroblastoma cell line (Schneider, 1976). Although butyrate appears to be specific in its actions on transformed cells, its effects on normal cells have not yet been examined.

2. Material and Methods

2.1. Animals

This study was carried out on 100 male albino rats of Sprague-Dawley strain (*Rattusnorvegicus*), their weight ranged between 150–170 g. During the study, the animals were housed in wire mesh cages and were fed standard rat chew and allowed free access to water. They were kept under constant environmental conditions [temperature (23±2°C), relative humidity (55 ± 5%) and light (12 h light dark cycles)]. All animals were weighed at the beginning and at the end of the study.

2.2. Experimental Design

The studied animals were randomly divided into the following groups:

Group I (control group): This group included 10 rats which were received intraperitoneal injection of saline.

Group II (Parkinson group): This group included 30 rats. Parkinson's disease (PD) was induced by intraperitoneal injection of Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) (ACROS ORGANICS, Belgium) in a dose of 10 mg/kg body weight (Thiruchelvam, et al. 2000) once weekly for 6 weeks.

Group III (Sodium Butyrate group): This group included 20 rats. Rats were intraperitoneal injected by sodium butyrate (Sigma-Aldrich St. Louis MO, USA) in a dose of 100 mM/kg (Sauer, et al. 2007) body weight three times weekly for6 weeks.

Group IV (PD co-administered with sodium butyrate group): This group included 20 rats. Parkinson's disease was induced by intraperitoneal injection of Paraquat (20%) in a dose of 10 mg/kg (Thiruchelvam, et al. 2000) body weight once weekly forat least 6 weeks and at the same time of PD induction, this group was co-administered by intraperitoneal injection of sodium butyrate in a dose of 100 mM/kg (Sauer, et al. 2007) body weight three times weekly for 6 weeks.

Group V (PD post-treated with sodium butyrate group): This group included 20 rats. Parkinson's disease was induced by intraperitoneal injection of Paraquat (20%) in a dose of 10 mg/kg (Thiruchelvam, et al. 2000) body weight once weekly forat least 6 weeks. After PD induction, this group was post-treated by intraperitoneal injection of sodium butyrate in a dose of 100 mM/kg body weight three times weekly for 6 weeks (Sauer, et al. 2007).

2.3. Behavioral Tests

The shuffling gait observed in PD patients can be considered analogous to forepaw stride length, which is also easily evaluated in models of PD. In this test, rat is trained to walk down a narrow corridor which was then lined with a clean white paper. The two front feet were then dipped into non-toxic ink, and the rat was allowed to walk through the corridor again. Once the footprint was dried, the paper was scanned and the distances between the footprints were measured (Tillerson*et al.* 2002 and Taylor *et al.* 2010).

2.4. Tissue Sampling

After scarification, brains were dissected carefully, washed three times with ice cold saline to remove extraneous materials, blotted individually on ash-free filter paper. The brain was chilled on ice and divided into four pieces that were wrapped in aluminium foil and stored at – 70 C° till used for preparation of tissue homogenates, nuclear and DNA extracts and histology.

2.5. Methods

2.5.1. Histological Method

Animals were scarified after 6 weeks and their brain were dissected, fixed in 10% formalin, histological examination of the brain tissues was carried out according to Bancroft and Stevens (1990) using Harris Hematoxylin and eosin staining technique.

- Procedure:

1) Paraffin sections were stained with Harris Hematoxylin for 5 min.

2) Sections were differentiated in 1% acidic ethanol for 5-30 seconds then Blueing agent was added.

3) Sections were stained with eosin for 2-5 minutes then washed by water until the desired shades of red or pink were obtained and then dehydrated in 90%, 96% and 100% ethanol for 5 minutes.

4) Sections were cleaned in two changes of xylene 5 minutes each or longer for better cleaning.

5) Sections were covered with clean cover slips then dried and microscopically examined. All stained slides were viewed by using Olympus microscope and images were captured by a digital camera (Cannon 620). Brightness and contrast were adjusted using Adobe Photoshop software (version 7.0; Adobe Systems, Mountain View, CA).

2.5.2. Immunocytochemical Method (HDAC-4 Anti-Body Stain)

Expression of HDAC-4 (clone: 9759) proteins was detected using avidin Biotin Complex (ABC) method (Ramprasad et al., 1996). The fixed midbrain sections from different group were examined by dewaxed and rehydrated sections were washed in distilled water for 5 min, rinsed in PBST for 10 min and incubated with 10% normal goat serum for 15 min to reduce non-specific background staining. Then, the sections were incubated with anti-rabbit HDAC-4 (Biovision, INC. USA) 1:100 for 1-2 hours at room temperature. The sections after 5

baths in PBST were incubated with biotinylated goat anti-rabbit immuoglobulin (Nichirei, Tokyo, Japan). The sections after 5 baths in PBST were further incubated with Avidin Biotin Complex (ABC: Nichirei, Tokyo, Japan) for 1 hour at room temperature. The reaction was developed by using 20 mg 3-3µ diaminobenzidinetetrahydrochloride (DAB, Wako pure chemical industries, Ltd) in 40 ml PBST, pH 7.2 containing 10 ml of hydrogen peroxide (H2O2) for 7-9 min at a dark room followed by distilled water then dehydrated and mounted. The prepared sections were examined by mean of a research microscope.

(i) Preparation of brain nuclear extracts: for estimation of total protein content & HDAC activity:

By using membrane, nuclear and cytoplasmic protein extraction kit supplied by Biobasic INC. Canada (Ouyang, et al. 2009).

- Principle:

The addition of solution A (cytoplasmic protein extraction) to the sample provides hypotonic condition that breaks cell membrane and releases proteins followed by centrifugation for collection of the nucleoli. Then, the nuclear proteins were extract by adding nuclear protein extraction solutiont B followed by centrifugation. An additional protease inhibitor cocktail and phosphatase inhibitor cocktail was included to maintain protein integrity and high activity. In addition, DTT helps to maintain the reduced state of the environment, by avoiding the false interaction between the cysteines, so as the protein will stay pure.

(ii) Determination of histone deacetylase (HDAC) activity in nuclear extract of brain tissue:

Using colorimetric HDAC activity assay Kit supplied by BIOVISION, USA.

- Principle:

First, the HDAC colorimetric substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity. Deacetylation of the substrate sensitizes the substrate, so that, in the second step, treatment with the Lysine Developer produces a chromophore. The chromophore can be easily analyzed using an an ELISA plate reader or spectrophotometer (Li, *et al.* 2012).

3. Results

3.1. Behavioral Tests Result

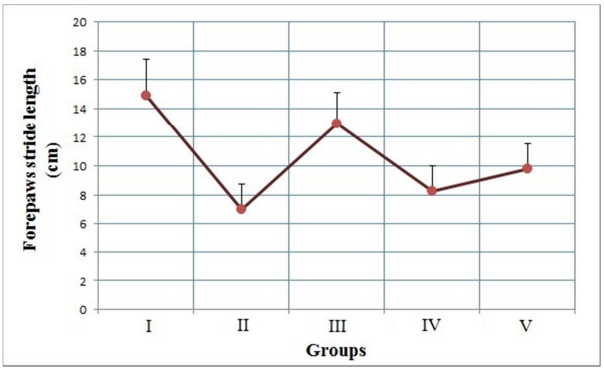

Fig. 3. *Forepaws stride length (cm) of the studied groups.*

Figure (3) shows a comparison of forepaws stride length among the five studied groups. PD group shows shortened stride length when compared with control one, while co and post treated groups shows normal stride length when compared with control and PD groups.

3.2. HDAC Activity

Figure (4) show a comparison of HDAC activity (O.D/ mg protein) among the studied groups. The HDAC activity is higher in PD group than the control and positive control groups. Also the HDAC activity is higher in co and post treated groups than the other studied groups. There is statistically significant difference between them ($P<0.01$, $P<0.001$).

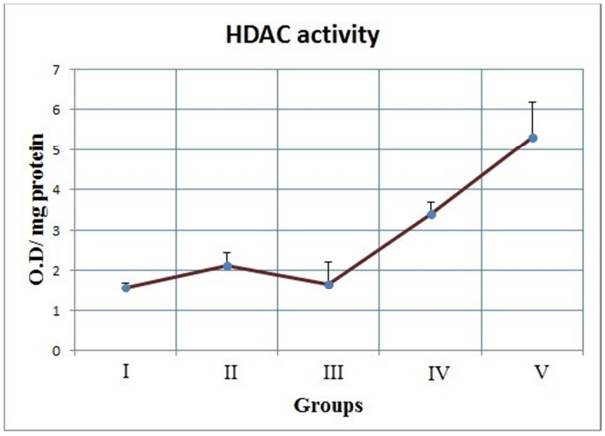

Fig. 4. *Histone deacetylase (HDAC) activity (O.D/ mg protein) of the studied groups.*

3.3. Histopathological Findings in the Brain

3.3.1. Light Microscope Investigations

Substantia nigra is a large mass of grey matter extending throughout the midbrain; on each side it divides the cerebral bundles into dorsal and ventral parts and in sections of the midbrain can be easily recognized by neurons containing grey pigment after which they got their name. The substantia nigra has extensive connections with the cortex, spinal cord, corpus striatum and reticular formation and appears to play an important part in the fine control of motor function. Control and butyric acid groups showed normal histological structures of the neurons and nerve fibers (Figs. 5 & 6)

The neurons of the substantia nigra are multipolar in form. In adults the cytoplasm contains numerous granules of neuromelanin pigment. The pigmented neurones of the substantia nigra contain dopamine which appears to act as neurotransmitter causing inhibitory effects particularly on neurones in the corpus striatum. Neuromelanin is contained in membrane-bound granules. Very little of these material is present at birth and increases gradually throw age.

Microscopic examination of the brain of Parkinson's group (G2) showed abnormal pallor of the substantia nigra correlating with loss of the pigment-containing nigralneurones and neuron degeneration (Fig. 7)

Microscopic examination of the brain of Co-administered

Parkinson's with butyric acid (G4) shows also abnormal pallor of the substantia nigra correlating with loss of the pigment-containing nigralneurones and neuron degeneration (Fig.8).

Post treated Parkinson's rat with butyric acid showed a moderate number of degenerating neurons in the midbrain with clear edema and vacuoles in substantia nigra (Figs.9).

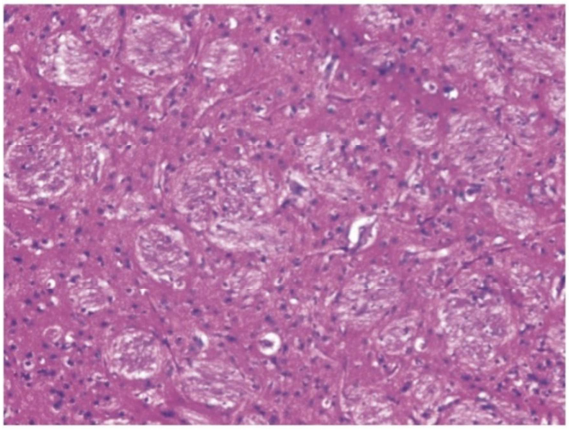

Fig. 5. *Micrograph of midbrain stained with H&E showing normal midbrain in control groups (X200).*

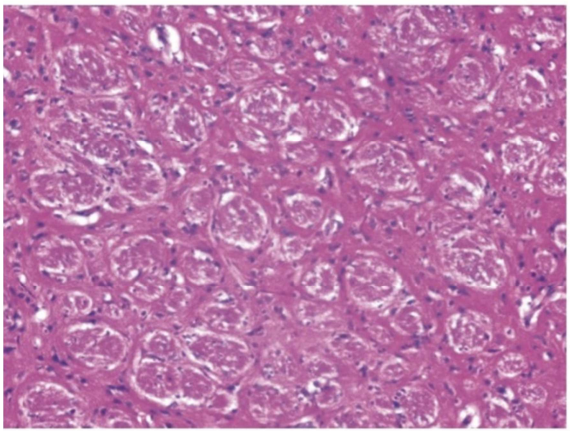

Fig. 6. *Micrograph of midbrain stained with H&E showing normal midbrain in positive control (Butyric acid) group (X200).*

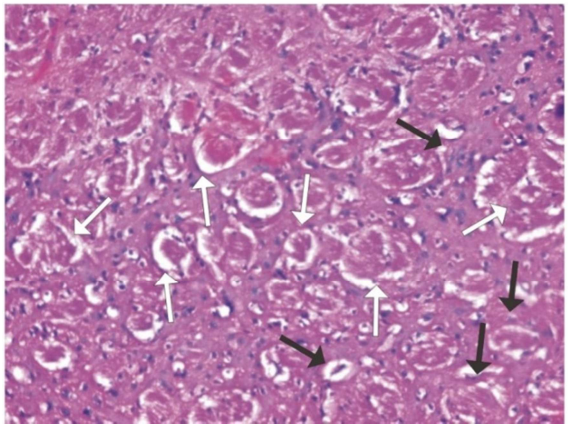

Fig. 7. *Micrograph of midbrain stained with H&E showing abnormal pallor of the substantia nigra in PD group, white arrows refer to degenerated neurons and black arrows refer to vacuoles (X200).*

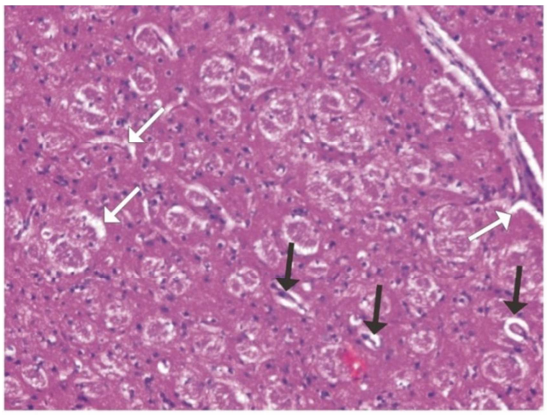

Fig. 8. *Micrograph of midbrain stained with H&E showing loss of the pigment-containing nigralneurones (black arrows) and neuron degeneration (white arrows) of co-administered (G4) group (X200).*

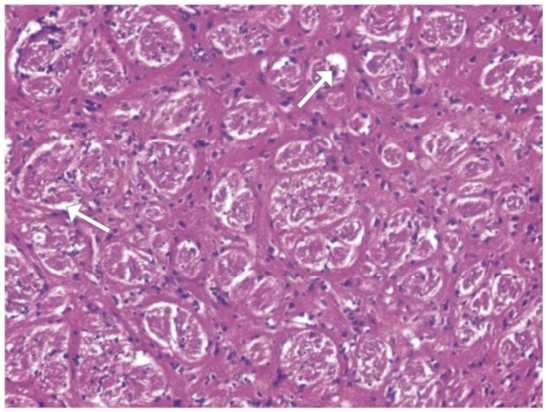

Fig. 9. *micrograph of midbrain stained with H&E showing a moderate number of degenerating neurons in the midbrain with clear edema and vacuoles (white arrows) of post-treated (G5) group (X200).*

3.3.2. Immunohistochemistry Investigation (HDAC-4 Anti-body Stain)

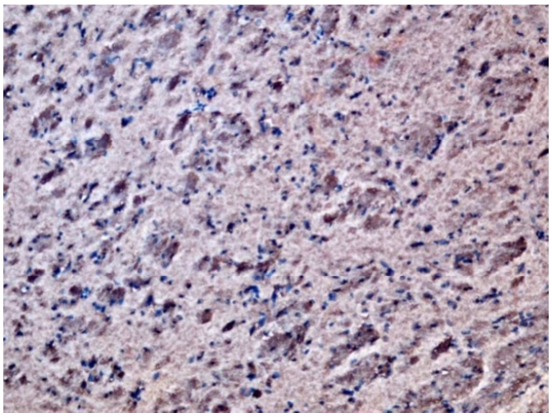

Fig. 10. *Photo micrograph of midbrain stained with HDAC anti-body in the midbrain of control group showing low reaction with HDAC stain (X200).*

Examination of brain sections in control group (G1) and butyric acid group (G3) stained by immunohistochemistry technique showed low affinity of HDAC stain was observed (Figs. 10, 11). Moderate affinity of HDAC stain was detected in the midbrain sections in Parkinson's disease group (G2)

(Fig. 12). Strong affinity of HDAC antibody stain was observed in the midbrain sections of Co-administered group (G4) (Fig. 13). The intensity of affinity of HDAC stain in Parkinson's brain section was increased when compared with control brain section. Very strong positive affinity for HDAC stain was observed in post-treated with butyric acid (G5) in brain sections (Fig. 14). Percentage of antibody-stain affinity among different groups is recorded in (Fig. 15).

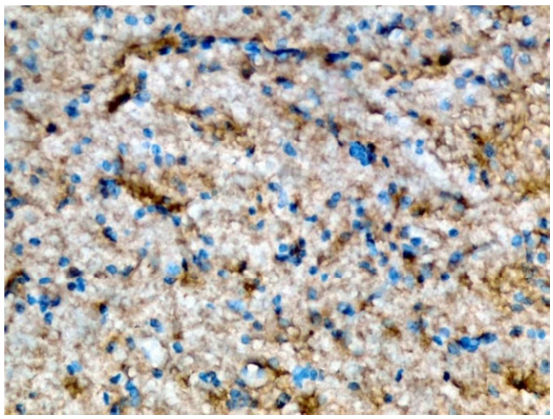

Fig. 11. *Photo micrograph of midbrain stained with HDAC anti-body in the midbrain of butyric acid group showing low reaction with HDAC stain (X200).*

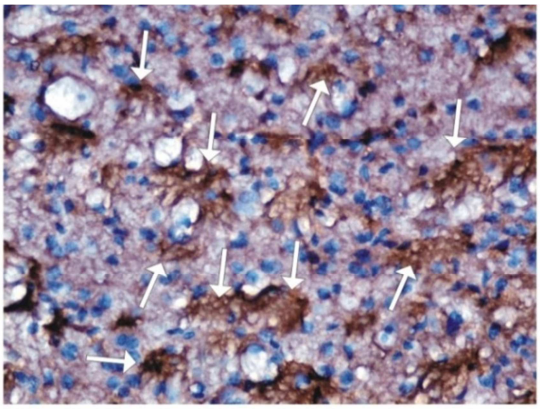

Fig. 12. *Photo micrograph of midbrain stained with HDAC anti-body in the midbrain of Parkinson's disease group showing moderate reaction (white arrows) with HDAC stain (X200).*

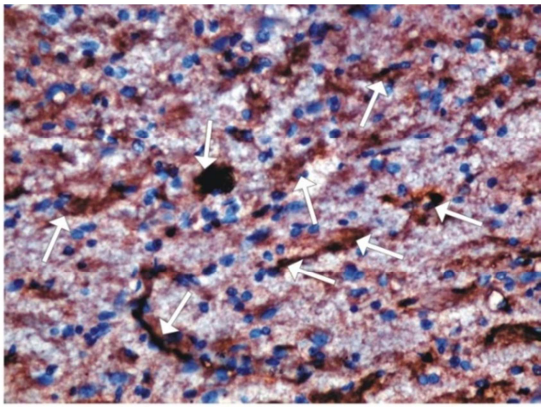

Fig. 13. *Photo micrograph of midbrain stained with HDAC anti-body in the midbrain of Co-administered (paraquate with butyric acid) group showing strong reaction (white arrows) with HDAC stain (X200).*

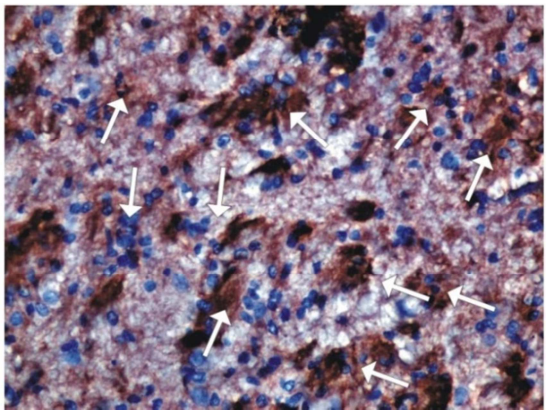

Fig. 14. *Photo micrograph of midbrain stained with HDAC anti-body in the midbrain of post-treated (butyric acid after paraquate) group showing very strong reaction (white arrows) with HDAC stain (X200).*

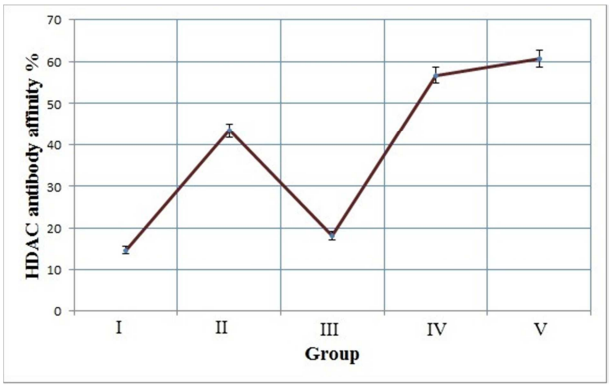

Fig. 15. *Showing the affinity percentage of HDAC antibody stain between groups.*

4. Discussion

Accumulated evidence suggests that Parkinsonism may arise from a combination of genetic susceptibility and exposure to environmental toxins. Indeed, several environmental risk factors such as metals, solvents, carbon monoxide and herbicides have been linked to the incidence and progression of PD (Tieu, 2011). Amongst these factors, the herbicide paraquat (PQ) shows clear neurotoxicity in the central nervous system. PQ can enter the central nervous system through neutral amino acid transporters associated with the blood brain barrier system (Shimizu et al., 2001) and selectively damage the substania nigra neurons in animal model (McCormack, et al., 2005).

The interest in PQ potential neurotoxicity began after the observation that paraquat exhibits a striking structural similarity to MPP+, the active metabolite of MPTP a neurotoxin that induces PD-like features in rodents, non-human primates and humans (Hisahara and Shimohama, 2010). Furthermore, epidemiological studies in agricultural communities have suggested an increased risk for PD due to paraquat use, raising the possibility that paraquat could be an environmental parkinsonian toxin (Moretto and Colosio, 2011). Paraquat rat model of PD also shows slow progressive loss of dopaminergic neurons and hence mimics what is seen in patients suffering from PD as one month in a rat's lifetime is

equivalent to 2.5 years in human (Muthukumaran et al., 2014).

In rodent models of PD, paraquat induced not only degeneration of the nigral dopaminergic neurons but also motor deficits mimicking clinical symptoms of PD (Peng, et al., 2004). Impairments in motor performance as displayed in PD patients can be monitored in rodent models by various behavioral tests (Taylor, et al., 2010). The current work used paw prints for the neurobehavioral assessment.

Gait disorders are common symptoms of PD. Typically, patients present a shortened stride length with a shuffling gait and reduced velocity (gait hypokinesia) (Lewis, et al., 2000). The present study demonstrates that a simple method using paw prints can be used in the rat to obtain consistent and easily measurable stride lengths. The method is sensitive to pharmacological and neurodegenerative alterations so it could be used as a reliable index of motor disorders due to basal ganglia dysfunction (Fernagut, et al., 2002). Further, the pole test has been known to be a very sensitive method that can detect nigrostriatal dysfunction (Hong, et al., 2014). This test has been used as a sensitive method to assess the ability of the rat to coordinate limbs to descend a pole from a fixed height (Meredith and Kang, 2006). Thus, it has been suggested that movement disorder in animal caused by striatal dopamine depletion can be detected by this test (Matsuura, et al., 1997).

The result of the present study showed significant decrease in the stride length in PD group when compared with controls and insignificant change in sodium butyrate treated group and significant increase in PD treated with sodium butyrate.

The significant changes in behavior as a result of sodium butyrate (SB) treatment can be explained as follows; sodium butyrate was shown to induce neuronal differentiation in primary rat cortical cultures, presumably by inducing the neurogenic basic helixloop-helix transcription factor Neuro D (Hsieh et al., 2004).

Also sodium butyrate has been shown to induce hippocampal neurogenesis in rats. It also stimulates neuronal differentiation of adult hippocampal neuronal progenitors (Hsieh et al., 2004). However, it is well documented that SB, is neuroprotective in both in vitro and in vivo experimental settings, (Hsieh et al., 2004).

The current study showed insignificant change in HDAC activity between sodium butyrate treated group and normal control group, this can be explained as follows: A term "butyrate paradox" was born to emphasize opposite effects of butyrate on the normal and neoplastic cells at the level of proliferation, differentiation, and gene expression (Mork et al., 2005).

However the current study showed significant increase in HDAC activity between PD groups treated with sodium butyrate and PD group, this can be explained as follows: sodium butyrate recently have been shown to be potent apoptosis inducers in a variety of cancer cells (Chen and Faller, 2005). SB induced apoptosis and its activity is mediated by increased acetylation of histones H3/H4, p53 and up-regulation of Bax (Chen et al., 2003).

Importantly, induction of Bax by SB appears to be cell type specific. SB induced Bax and down-regulated Bcl2 level, a critical level of HDAC1 is important to maintain cellular homeostasis, a twofold increase of HDAC1 was sufficient to confer resistance to SB-induced apoptosis. (Sibaji et al., 2011).

Also, it was showed that increased resistance to apoptosis in HDAC1-overexpressing clones was associated with impaired Bax expression. Overexpression of all three members of the class I HDAC family (HDAC1, HDAC2, and HDAC3) repressed Bax promoter activity, whereas inhibition of HDAC activated it (Sibaji et al., 2011). Down-regulation of p53 function relies largely on the COOH-terminal region of p53 containing the basic lysine residues Lys-373 and Lys-382. SB increased p53 acetylation at Lys-373 and/or Lys-382 (Sibaji et al., 2011).

Since acetylation of p53 by p300 greatly enhances its DNA-binding ability and hence the transactivation activity, it is very likely that the observed loss of p53 transactivation activity is due to direct deacetylation of p53 by HDACs. It is reasonable to speculate that HDACs directly deacetylate p53 at its C-terminal domain and thus alleviate its ability to activate gene (Berger, 2007). P53 may form a complex with HDACs via direct binding; deacetylase activity is indeed required for HDACs to fully repress p53 function. P53 could be as an equally good substrate as histone H4 for HDACs, p53 is a physiological substrate for histone deacetylases (Li-Jung et al., 2000).

In co-administered and post-treatment groups, sodium butyrate unexpectedly did not show inhibitory effect on HDACS activity, moreover , HDACS activity remained elevated compared to the control group and in parallel with the paraquat group, there are possible explanations for these results.

Firstly , the majority of HDAC inhibitors that are currently either in clinical testing or that are on the market target multiple isoforms of the classic HDAC family (classes I, II, and IV) but do not inhibit SIRT family members. Short Chain Fatty Acids, these inhibitors include compounds with rather simple structures, such as valproic acid, phenyl butyrate, and butyrate. Short-chain fatty acid inhibitors show similar profiles in terms of their action at class I and IIa HDACs with some differences detected in terms of individual potencies in vitro. They tend to be less potent in inhibiting HDAC activity than hydroxamic acids (millimolar compared with Nano molar range) (Grayson et al., 2010). Among these, sodium butyrate has diverse properties and its clinical development has been hampered by its short half-life and difficulty in achieving millimolar levels in vivo (Foglietta et al., 2014).

Secondly, it is well known that HDAC1 Protects from DNA damage, sustains DNA damage checkpoint, maintains DNA replication and regulates oxidative stress (Zupkovitz et al., 2010). Overexpression of Histone Deacetylase 1 confers resistance to Sodium Butyrate through a p53-mediated pathway. HDAC 1 has antagonistic effect for sodium butyrate. Luciferase assays also showed that overexpression of all three members of the class I HDAC family HDAC1, HDAC2, and HDAC3 reduced the potency of sodium butyrate (Bandyopadhyay et al., 2004). Also, it was seen that the

strongest increase was for HDAC5 and 6, a three to four-fold increase both after and butyrate treatment (Ajamian et al., 2004).

Moreover, similar to HDAC6, HDAC10 also shows resistance to sodium butyrate. At least two possibilities can explain this unique pharmacological property of HDAC6 and -10. First, their common N-terminal catalytic domain might specifically confer resistance to sodium butyrate Alternatively, the presence of the second complete (in HDAC6) or incomplete (in HDAC10) catalytic domain renders this subfamily resistant to SB (Guardiola andYao, 2002). Collectively, oxidative stress is caused by paraquat and to ameliorate the oxidative damage , HDACS rise to counteract cellular stress and DNA damage .Although sodium butyrate can inhibit some subclasses of HDACS , it is unable to inhibit other groups of HDACs such as HDACs 1,HDAC6 and HDAC 10 and the class III HDACS , On the other hand, sodium butyrate was able to increase the activity of some HDACS such as HDAC 5.these results explain the diverse action of sodium butyrate on HDACS and that different HDACs are regulated differently by HDAC inhibitors, suggesting differential sensitivity and roles for the individual enzymes. These above mentioned data are positively correlated with our results that showed significant increase in HDACS activity in the co-administered and post-treatment groups.

Finally, oxidative stress is one of the factors regulating the acetylation of histone by increases HDAC level and activity (Miura et al., 2008 and Yao et al., 2013).

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Abbreviations Used

PD: Parkinson's disease, ip: Intra peritoneal, HDAC: Histone deacetylase, MPTP: Methy phenyl tetrahydro pyridine, HAT: Histone Acetyl Transferase, SB: Sodium butyrate.

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