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Epigenetic Study of Parkinson's Disease in Experimental Animal Model

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Abstract Epigenetic modifications are defined as mechanisms that are able to modify the expression levels of selected genes without necessarily altering their DNA sequence, as histone tail modifications. 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 epigenetic fingerprint in PD experimental model through HDAC, PARP and activities oxidative stress markers. **Materials and methods:** The study was carried out on five rat groups, control group, Parkinsonism group, sodium butyrate group, two parkinson's disease groups co-treated and post treated with sodium butyrate. Parkinsonism was induced by ip injection of paraquat. Laboratory measurements included serum 8-OHdG, MDA level as biomarkers of oxidative stress. HDAC and PARP activities were measured as other epigenetic mechanisms. **Results:** PD group, PD co-treated and post treated with sodium butyrate showed significant increase in HDAC and PARP activities. Also, there was significant increase in serum 8-OHdG level and MDA level in both serum and tissue. **Conclusion and recommendations:** The increments in HDAC and PARP activities are either two 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.

Keywords: *Parkinson's disease, Oxidative stress, HDAC, 8-OHdG, Sodium butyrate*

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1. Introduction

Epigenetics is more accurately defined as the study of any potentially stable and, ideally, heritable change in gene expression or cellular phenotype that occurs without changes in Watson- Crick base-pairing of DNA. [1] Several changes to the genome fall into the category of epigenetics, including DNA methylation, histone modifications, chromatin remodeling and micro RNA (miRNA) [2].

Parkinson's disease (PD) also known as idiopathic or primary Parkinsonism, hypokinetic rigid syndrome/HRS, or paralysis agitans is the second most common neurodegenerative disorder affecting about 0.3% of the entire population and 1% of people over 60 years of age. [3] Aging is the major known risk factor for PD. The aging process is characterized by a loss of phenotypic plasticity of the cells to respond to deleterious effects. Consequently, cells are exposed to an increased amount of oxidative stress, perturbed energy homeostasis, accumulation of damaged proteins and lesions in their nucleic acids [4]. Also, 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. [5].

Classically, PD is considered as a movement disorder, and its diagnosis is based on the presence of a set of cardinal motor signs (e.g. rigidity, bradykinesia, rest tremor, and postural reflex disturbance and gait disturbance [6]. These symptoms of PD mainly result from the progressive and profound loss of neuromelanin-containing dopaminergic neurons in the substantianigra pars compacta (SNpc) that leads to a loss of dopamine, its metabolites, its biosynthetic enzyme [tyrosine hydroxylase (TH)] and the dopamine transporter (DAT) in the striatum, as well as in the SNpc [7].

Cognitive impairments in PD are often associated with the deposition ofLewy bodies (LB) and Lewyneurites (LN) in various areas of the brain, including cortex and midbrain. Lewy bodies are filamentous protein inclusions

formed by abnormal cytoplasmic eosinophilic aggregates of α-synuclein, parkin, ubiquitin, and other proteins [8].

Epigenetics, which refers to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure, is acquired throughout life and depends upon environmental clues, such as lifestyle, diet and toxin exposure. Consequently, since PD is age-related complex disorder, epigenetics might be the missing link between environmental risk factors and the sporadic form of neurodegeneration [9].

In addition to epigenetic modifications, increasing evidence indicates that multiple biochemical and cellular factors are involved in neuronal death in PD, some of them involve protein dyshomeostasis, mitochondrial impaired function [10]. These processes contribute to the oxidative stress and damage and inflammatory response in brain of PD. The current belief is that dopaminergic neuronal degeneration in PD is likely to result from a combination of multiple interlinking signaling pathways rather than from a single unifying mechanism [11].

Neurochemically, PD is characterized by mitochondrial dysfunction and brain mitochondrial oxidative damage. There are also consistent observations of the impaired functioning of mitochondrial respiratory transport chain at the site of complex I (NADH CoQ10 reductase), from PD brain, particularly in the SNpc, with consequent aggregation and accumulation of α-synuclein. [12] The deficiency of complex I in PD could result from either an environmental toxin or an acquired or inherited mitochondrial DNA mutations [13].

Inhibition of complex I create an environment of oxidative stress that ultimately leads to the aggregation of α-synuclein with the consequent neuronal death. [14] Complex I dysfunction results in complex I inactivation, reduced oxygen (O_2) uptake and ATP formation, increased O_2^- formation, oxidative stress and lipid peroxidation, events that lead to neuronal depolarization and contribute to excitotoxic neuronal injury. Further, Somatic mitochondrial DNA mutations have been reported in PD brain [15].

Among the various accepted experimental modelsof PD, neurotoxins have remained the most popular tools to produce selective neuronal death in both *in vitro* and *in vivo* systems. [16] The key neurotoxic models of PD, namely those produced by the toxins 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), rotenone, and paraquat (PQ) [17].

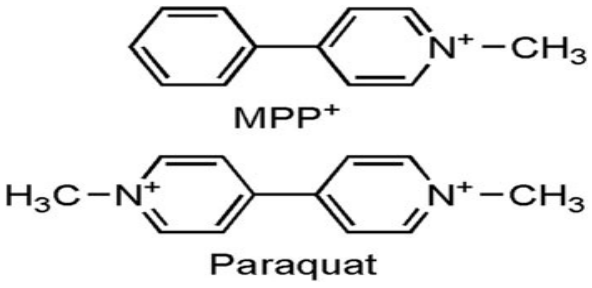

Figure 1. Comparison of chemical structures of MPP+ and paraquat [19]

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 [18]. 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 (Figure 1) [19].

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 [20].

Exposure to paraquat has been shown to induce proteasome dysfunction, α-synuclein aggregation, and potentiation of α-synuclein-induced toxicity. [21] It has been hypothesized that mutated α-synuclein induces ROS generation and initiation of the apoptotic cascade, a reduction in vesicle number and accumulation of cytoplasmic dopamine. In the cytosol, dopamine is metabolized by monoamine oxidase which generates H_2O_2, or is auto-oxidized generating O_2^-, H_2O_2, and dopamine-quinone species [22].

Thus, paraquat induces oxidative stress reflected as the accumulation of reactive oxygen species (ROS), lipid peroxidation and DNA damage. In general, herbicides have been shown to alter cellular redox balance by different mechanisms including: (1) their enzymatic conversion to secondary reactive products and/or ROS; (2) depletion of antioxidant defenses; and (3) impairment of antioxidant enzyme function [23].

Histone deacetylases (EC3.5.1.98, HDAC) are a class of enzymes that remove acetyl groups (O=C-CH_3) from an ε-N-acetyl lysineamino acid on a histone, allowing the histones to wrap the DNA more tightly. This is important because DNA is wrapped around histones, and DNA expression is regulated by acetylation and de-acetylation. Its action is opposite to that of histone acetyltransferase. HDAC proteins are now also called lysine deacetylases (KDAC), to describe their function rather than their target, which also includes non-histone proteins [24].

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, [25] DNA replication and cell cycle progression. 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 [26].

Progression through the cell cycle is dependent on expression of cell cycle regulating proteins that are involved in cell growth and DNA replication. Members of the E2F family of transcription factors have been shown to possess important and conserved roles in cell cycle progression in several organisms examined. [27] Eight members of the E2F family of proteins have been characterized to date. [27] E2F-1 activation has been established as a contributing factor in a number of neurodegenerative *in vitro* and *in vivo* models.

It has been shown that some E2F family members including E2F-1 are acetylated by p300 and CBP. P300 and CBP are HATs that counteract HDAC function in the cell [28]. Acetylation of E2F-1 was shown to enhance

transactivation of an E2F responsive promoter. However, the reversal of this acetylation by HDAC1 suggests that HDAC activity can serve as a mechanism in controlling the cell cycle. In addition, another group reported that HDAC1 is present in Rb-recruited transcription repression complexes. Nicolas et al., (2000) showed that RbAp48, an Rb-associated protein that can directly interact with histone H4, and E2F-1 associate directly in the presence of Rb and HDAC1 to mediate transcriptional repression [29].

Another HDAC whose role in regulation of cell cycle has been uncovered is HDAC3. It undergoes a caspase dependent degradation during apoptosis. The degradation of HDAC3 might be the underlying reason for the instability of E2F-responsive elements in apoptotic versus neuroprotective conditions [30].

Forty years ago, in their initial paper entitled ‘‘Nicotinamide mononucleotide activation of a new DNA-dependent poly adenylic acid synthesizing nuclear enzyme’’ Chambon, Weiland Mandel correctly anticipated the major property of an enzymatic activity involved in DNA-dependent NAD consumption. This seminal contribution triggered the science of poly (ADP-ribose) (PAR) that today covers a large area of biology dealing with genomic stability and energy metabolism. Poly(ADP-ribosyl)ation of nuclear proteins is a post-translational modification induced by DNA strand breaks that establishes a molecular link between DNA damage and chromatin modification. A large body of evidence demonstrates that this activity efficiently contributes to a detection signaling pathway leading ultimately to the resolution of strand-break interruptions. In certain pathophysiological conditions, this protecting function is strongly activated leading to cell death, tissue damage and organ failure [31,32].

PARP-1 contributes in many unique ways to the molecular biology of nuclear processes, playing key roles in the maintenance of genomic integrity, the regulation of chromatin structure and transcription, and the establishment of DNA methylation patterns, as well as a host of other processes (e.g., mitotic apparatus function, cell death pathways) [33].

Butyric acid is a fatty acid occurring in the form of esters in animal fats and plant oils. The triglyceride of butyric acid makes up 3% to 4% of butter. When butter goes rancid, butyric acid is liberated from the glyceride by hydrolysis leading to the unpleasant odor. It is an important member of the fatty acid sub-group called short-chain fatty acids. Butyric acid is a weak acid with a pKa of 4.82, similar to acetic acid which has pKa 4.76. [34] The similar strength of these acids results from their common -CH_2COOH terminal structure. [35] Pure butyric acid is 10.9 molar.

The role of butyrate changes depending on its role in cancer or normal cells. This is known as the "butyrate paradox". Butyrate inhibits colonic tumor cells but promotes healthy colonic epithelial cells, but the signaling mechanism is not well understood. [36] The chemopreventive benefits of butanoate depend in part on amount, time of exposure with respect to the tumorigenic process, and the type of fat in the diet. Low carbohydrate diets like the Atkins diet are known to reduce the amount of butyrate produced in the colon [37].

Roles for butyrate have been established in a number of epigenetically controlled activities such as cell differentiation, proliferation, motility, induction of cell cycle arrest, apoptosis [56], and even in memory formation. However, the mechanisms by which butyrate suppresses growth and induces cellular differentiation or apoptosis are not known in details [38]. Microarray assays of global gene expression profiles have shown that over 450 genes were significantly regulated by butyrate in bovine kidney epithelial cells. Most of them were down-regulated, but over 30 genes were up-regulated [39]. Among the down-regulated genes were genes crucial for initiation of DNA synthesis such as MCM and Orc proteins, which are essential for the assembly of the prereplication complex. CDC2/Cdk1 and related cyclins were also down-regulated. On the other hand, genes related to apoptosis were up-regulated. In another assay over 10,000 genes were found responsive to butyrate regulation in human epithelial cells. Butyrate exerts several Modulatory effects on nuclear proteins and DNA such as induction of histone acetylation and phosphorylation, and hypermethylation of cytosine residues in DNA. The steady state of histone acetylation is controlled by the equilibrium of two distinct families of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) [39].

2. Materials and Methods

2.1. 1-Animals

This study was carried out on 100 male albino rats of Sprague-Dawley strain (*Rattus norvegicus*), 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 (Acros organics, Belgium) in a dose of 10 mg/kg body weight [40] 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 [41] body weight three times weekly for at least 6 weeks.

***Group IV (PD co-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 [40] body weight once weekly forat least 6 weeks and at the same time of PD induction, this group was co-treated by intraperitoneal injection of sodium butyrate in a dose of 100 mM/kg [41] body weight three times weekly forat least 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 [40] body weight once weekly for at least 6 weeks. After PD induction, this group was post-treated by intraperitoneal injection of sodium butyrate in a dose of 100 mM/kg [41] body weight three times weekly for at least 6 weeks.

*At the end of the experiment, animals will be evaluated for the development of the disease by the following behavioral tests:

The shuffling gait observed in PD patients can be considered analogous to forepaw stride length [42].

Pole test: for motor coordination/nigrostriatal dysfunction of rat [43].

Physical Phenotype Assessment: The rats were evaluated for their body position [44].

All experiments were carried out according to the guidelines of the Ethical Committee of Tanta University, Faculty of Science.

2.3. All groups were subjected to the following

1. Determination of serum Oxidative DNA damage (8-hydroxy-2'-deoxyguanosine) by ELISA kit (Enzo life sciences, USA) [45].

2. Colorimetric determination of:

a) Serum and brain malondialdehyde (MDA) level (Sigma-Aldrich St. Louis MO, USA) [46].

b) Histone deacetylase (HDAC) activity in nuclear extract of brain tissue (Biovision, USA) [47].

c) Poly (ADP) ribose polymerase enzyme in nuclear extract of brain tissue (Trevigen, USA) [48].

3. Results

Figure 1 shows a comparison of the forepaws stride length in centimeters among the five studied groups. The values are significantly decreased in PD rats (G II) compared to control one. While the values in positive control group (G III) showing no deference compared to control one. The values in co-treated and post treated (G IV, G V) groups are significantly increased compared to PD rats (G II).

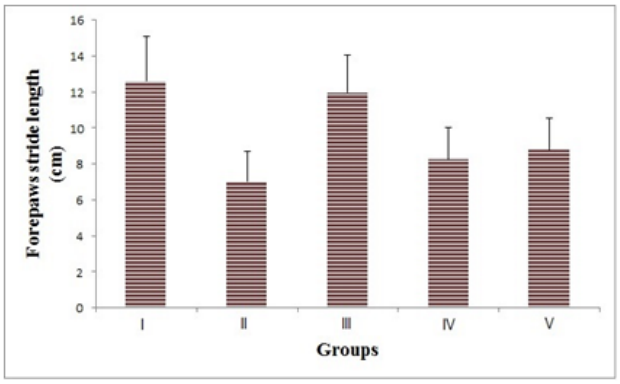

Figure 1. Forepaws stride length (cm) of the studied groups.

Table 1. Forepaws stride length and pole tests for the neurobehavioral assessment and body posture and tail positions f or the physical phenotype assessment of the two studied groups

Test	Control group	PD group	Co-treated group	Post treated group
Forepaws stride length	Normal stride length	Shortened stride length	Normal stride length	Normal stride length
Pole test	Negative	Positive*	Positive*	Negative
Body posture	Normal elongated	Curved (crouched)	Semi Curved (crouched)	Normal elongated

*positive pole test (for Parkinsonism) =ratdropped from thepole

Table 1 shows a comparison of forepaws stride length and pole tests for the neurobehavioral assessment and the body and tail positions for the physical phenotype assessment 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, curved (crouched) posture and dropped from the pole however is not evident.

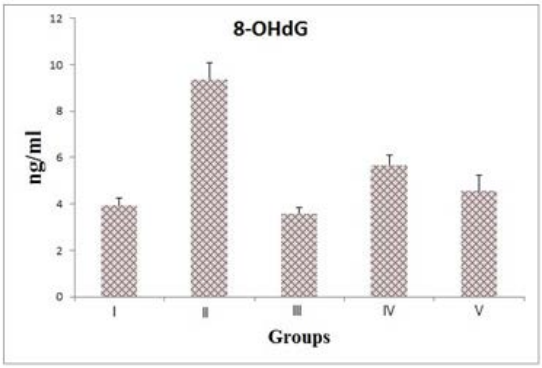

Figure 2. Serum 8-OHdG level (ng/ml) of the studied groups

Figure 2 show a comparison of serum 8-hydroxy-2'-deoxyguanosine level (ng/ml) among the studied groups. The values are significantly increased in PD rats compared to control and positive control groups. While the values are significantly decreased in co and post treated rats compared to PD one. There is statistically significant difference between them ($p< 0.001$).

Figure (3a&3b) shows a comparison of serum MDA level (nmol/ml) and brain MDAlevel (nmol /g tissue) among the studied groups. The serum and brain level of MDA are significantlyhigher in PD rats compared to control and positive control groups. On the other hand the serum and brain level of MDA are significantly lower in co and post treated rats compared to PD group. There is statistically significant difference between them ($P<0.001$, $P<0.01$, $P<0.001$) respectively.

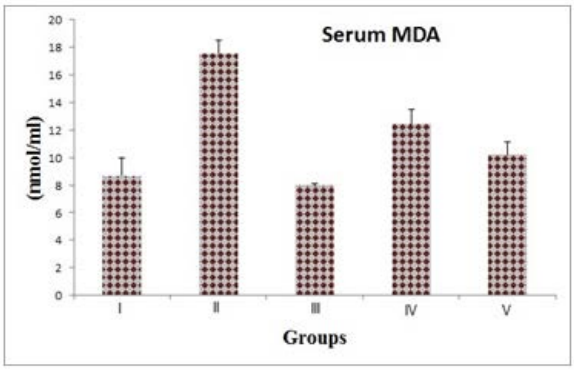

Figure 3 (a). Serum MDA level (nmol/ml) of the studied groups

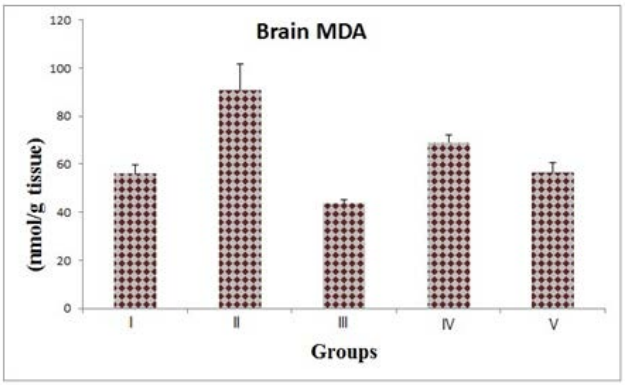

Figure 3 (b). Brain MDA level (nmol/g. tissue) of the studied

Figure 4 shows 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). Figure 5 show a comparison of poly (ADP-ribose) polymerase activity (U/ mg protein) among the studied groups. The PARP activity is higher in PD group than the control and positive control groups. Also the PARP 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).

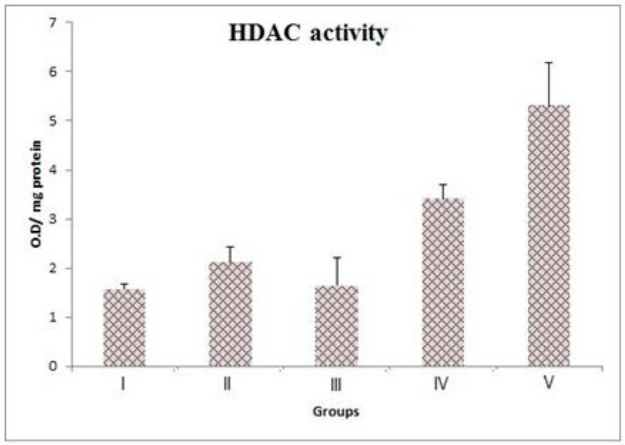

Figure 4. Histone deacetylase (HDAC) activity (O.D/ mg protein) of the studied groups

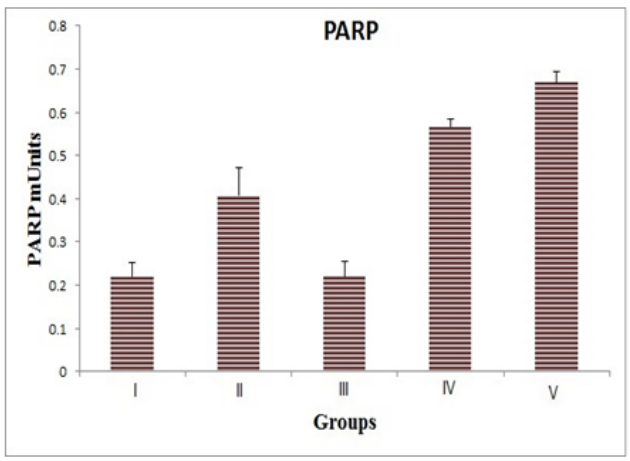

Figure 5. poly (ADP-ribose) polymerase (PARP) activity (U/ mg protein) of the studied groups.

4. Discussion

Induction of PD by using paraquat was documented by changes in the weight and behavioral status of the treated animals. The present study showed that there were statistically significant differences in the body weight between the all studied groups at the end of the experiment. The published results of Cicchetti et al. [50] were not far from the current study as most of the animals treated with PQ or a combination of herbicide/fungicide (PQ/Maneb) progressively gained weight, albeit more slowly than vehicle-treated animals. Moreover, Chanyachukul et al. [51] documented that significant reductions in body weight gain of PQ at a dose of 10 and 20 mg/kg were observed. In addition, no significant effect was found in feeding, drinking and ability of the animals to maintain normal growth and full health.

The etiology of reductions in body weight gain in PD group may be related to various factors. Systemic PQ administration was followed by both noradrenergic and dopaminergic disturbance. The alteration of norepinephrine in the hypothalamus may be attributed to PQ effect that derived from its distribution in the poor or weak BBB areas, since ventral hypothalamus, anterior olfactory bulb, and the medulla oblongata are known to have improper BBB. This effect may be linked to a reduction in feeding and body weight control systems in the brain [52].

Consistently, weight gain reduction in the current study can be explained by the notion that dysphagia caused by oropharyngeal dysfunction and hyposmia, which could be responsible for weight loss, are common findings in advanced PD patient. [53] Also, lesions similar to the ones observed in the brain have been identified in the submucosal plexus of the enteric nervous system on routine colonic biopsies of PD patients. In addition, constipation and gastric emptying problems are associated with PD that caused by degenerations of the dorsal vagal nucleus and the intramural plexus of the whole intestine, which probably develop prior to the degeneration of dopaminergic neurons of the substantianigra [54].

In the present study there were significant increase in body weight gain in all sodium butyrate treated groups. These results can be explained as follow, sodium butyrate induce absorption of water and sodium and proliferation of intestinal cells resulting in a larger absorptive surface, also it is used as energy resources and stimulate intestinal blood flow and the synthesis of gastrointestinal hormones. [55] Butyric acid was also shown to induce cell differentiation and to regulate the growth and proliferation of normal colonic and ileal mucosa, Sodium butyrate and supplementation were also shown to improve body protein percentage [56].

Sodium butyrate was also shown to increase both blood glucose level and intestinal glucose absorption. This concurs with Zhanguo et al. [57] who reported also that, supplementation of butyrate improved serum glucose. The mechanism of butyrate action is related to promotion of energy expenditure and induction of mitochondria function [58].

This increase in intestinal glucose absorption may also be discussed as follows. The jejunum is the main site of glucose absorption. The sugar is transported across the mucosal membrane by a sodium-dependent secondary

active process that relies on the sodium gradient established by the Na+-K+ ATPase also Known as Na+-K+ pump [59].

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. In addition, PQ treated animals fail to descend or fall from the pole which is indicative of poor motor coordination. Further, PD animals show crouched (curved) posture compared to normal elongated posture of the control animals. This abnormal posture resembles the stooped posture of patient suffering from Parkinsonism that is caused by many factors including spinal rigidity, loss of normal subconscious posture control, poor balance, and a loss of normal proprioception. [60] Although the lesioned animals exhibited clear motor symptoms, such as crouched posture and short strides, the straub rigid tail of the PD group is not evident in this study. The results presented herein suggest that the administration of PQ to rat analyzed with behavioral tests shows an adequate validity as an animal model for the study of the motor signs and the physical phenotype of PD [61]. Sodium butyrate treated group showed insignificant change in descending or falling from the pole and posture curvature when compared to normal animals. Although PD groups treated with sodium butyrate showed significant changes in descending or falling from the pole and posture curvature when compared to PD group.

The significant changes in behavior as a result of sodium butyrate (SB) 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 [62].

Also sodium butyrate has been shown to induce hippocampal neurogenesis in rats. It also stimulates neuronal differentiation of adult hippocampal neuronal progenitors through induction of Neuro D. However, it is well documented that SB, is neuro protective in both in vitro and in vivo experimental settings. [63] The main mechanism underlying PQ toxicity is oxidative stress due to over production of ROS. Redox cycling and consequent ROS generation are thought to be key cytotoxic mechanisms induced by PQ [64].

In the present study a significant increase in both serum and brain MDA levels, as a marker of lipid peroxidation, in PQ treated group compared with the control group. The increment of MDA level could be explained on the base that initiation of the membrane destructive process of lipid peroxidation is a possible result of the cyclic reduction-oxidation of paraquat in biological systems [64]. Concomitantly, tissue levels of MDA in this study are positively correlated with those of serum concentrations. So serum MDA may be used as a marker for diagnosis and follow-up of Parkinson disease.

Clearly there are a large number of pathological conditions in which oxidative stress may have a role. At the very least, biomarkers of oxidative DNA damage offer the means by which this stress may be monitored, but they may have the potential to act as markers of disease development risk and to assess efficacy of therapy. Although ROS can damage a variety of cellular components, DNA is a critical target because it can lead to base modifications, basic sites and double strand breaks, all of which can alter the information content of cells [65].

The significant increase in the serum level of 8-OHdG in the PD group can be explained on the base that PQ is absorbed into the body and ROS are produced to attack DNA bases. Among ROS, the highly active hydroxyl radical can break the strands in DNA by reacting with C-8 position of the guanine via hydroxylation and generating 8-OHdG. In addition, nitric oxide can also react with superoxide to form peroxynitrite and thus produce 8-OHdG driven by a hydroxyl-radical-like mechanism [66]. As 8-OHdG is soluble in water, it is secreted out of the cell after being removed from the DNA helix. Thus, monitoring extracellular 8-OHdG can provide insights of oxidative DNA damage [67]. Oxidation occurs most readily at guanine residues because its high orbital energy that leads to high oxidation potential of this base relative to cytosine, thymine, and adenine resulting in accelerating generation of 8-OHdG. Because 8-OHdG is a product of oxidized DNA indicating oxidative cellular damage, such aberrant 8-OHdG levels may signal increased oxidative stress that could initiate a cascade of cell death events as a prelude to dopaminergic depletion. This observation implies that measuring 8-OHdG may serve as a marker for oxidative DNA damage in this animal model of PD [68].

It is also suggested that intracellular aldehydes, such as MDA, can directly react with DNA. These aldehydes can also react with some amino acids in DNA repair proteins and destroy protein function. Therefore, lipid peroxidation products may reduce DNA repairing capacity. Although some researchers suggested that 8-OHdG formation may be related to lipid peroxidation. [69] Thus, it may be inferred that increased level of oxidatively modified DNA and lipids observed in the present study creates a picture of increased oxidative stress in the system.

In the present study there were insignificant changes in oxidative stress markers in sodium butyrate treated group when compared to normal animals. Although there were significant changes in oxidative stress markers in the two PD groups treated with sodium butyrate. SB significantly inhibited the oxidative Stress and improved antioxidant capacity, these results were in accordance with that of Zhang et al. [70] Also sauer et al. [71] found that butyrate could enhance catalase activity in human colon cells in vitro, also Vanhoutvin et al. [72] showed that locally administered butyrate could significantly increase GSH-Px expression. SB exhibited increased percentage of arachidonic acid and total n-3 fatty acid contents [73].

Also, Abrahamse et al. [74] showed that butyrate is able to reduce DNA damage. The mechanism by which butyrate reduces DNA damage is not known. However, the simple scavenger activity of butyrate against oxygen free radicals is probably not the main mechanism. In addition, the chemical structure of butyrate makes the simple action as scavenger improbable. In this content, it is important to consider that DNA damage represents a steady-state between the initiation of DNA damage and its repair by cellular processes. Thus, DNA damage depends on protective endogenous factors, such as DNA repair and levels of antioxidant systems, which could be modified by the treatment with butyrate. The effects of butyrate on the chromatin structure and on the DNA repair mechanisms are known. [75] Butyrate is able to increase both chromatin accessibility to DNA repair enzymes and DNA

excision repair. These activities could result in a reduction of DNA damage, butyrate could affect the intracellular antioxidant enzymes (i.e. catalase and glutathione peroxidase) responsible for the reduction of H_2O_2 [76].

Neuroinflammation and oxidative stress are two hallmarks of neurodegenerative disorders. Therefore, identifying drugs to attenuate the production of proinflammatory molecules and ROS is an important area of research as such drugs may stop or delay the progression of neurodegenerative disorders. [77] SB inhibits the expression of various proinflammatory molecules (iNOS, TNF-α and IL-1β) from activated glial cells suggesting that SB is anti-inflammatory. On the other, it suppresses the production of ROS from microglial cells in response to various stimuli indicating its antioxidant activities [77].

Butyrate has additional metabolic effects. It can be a chaperone molecule. As a chemical chaperone, butyrate can bind and mask surface-exposed hydrophobic segments of unfolded proteins and thereby stabilize protein structure in the native conformation, reducing endoplasmic reticulum (ER) stress. Butyrate directly stabilizes mutant α-synuclein and prevents the formation of high molecular weight oligomers and fibrils [78],

A novel mechanism that may be involved in motor neuron death and neurodegenerative diseases is transcriptional dysfunction, which consists of aberrations of the molecular machinery that regulates gene expression through the manipulation of epigenetic markers. [79] The histone acetylation is the main type of covalent histone modification. HATs and HDACs act in an opposing manner to control the acetylation state of histones and non-histone proteins.

The current work elucidated a significant increase in HDAC activity in PQ treated group compared with the control one. This increase could have more than one possible explanation. Firstly, HDACs play an important role in oxidative DNA damage response as damage signaling involves phosphorylation of histones by ATM/ATR kinases. This is followed by chromatin opening and the involvement of H3/H4 acetylation, via HATs. Chromatin restoration after repair involves dephosphorylation by phosphatases and deacetylation of H3/H4 lysines by HDACs. [80] These data led to the “access-repair-restore” model whereby chromatin would be altered to allow access and repair of lesions followed by a restoration of chromatin organization. [80] This explanation is supported by the significant positive correlation that was observed between HDAC activity and 8-OHdG level in the current work.

Secondly, paraquat (PQ) induces acetylation of histones in dopaminergic neuronal cells and that the hyperacetylation can contribute to PQ-induced apoptosis [81]. These findings suggest that PD may be a disease of aberrantly increased histone acetylation and overexpression of HDAC could rescue against DNA damage and neurotoxicity [81]. Thus, HDACs activity increases to induce cell cycle arrest and resistance to oxidative stress. HDACs protect against apoptosis as they have been shown to deacetylate the promoter region of pro-apoptotic genes like c-Jun and P53 and thereby to inactivate their expression [82]. Likewise, HDAC has been demonstrated to be recruited by E2F4, a member of the E2F family of transcriptional regulators, to the promoter region of the proapoptotic gene B-myb, which was transcriptionally silenced upon being deacetylated. [82] Finally, oxidative stress is one of the factors regulating the acetylation of histone by increases HDAC level and activity [83].

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. [84] Although the current study showed significant change 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. SB induced apoptosis and its activity is mediated by increased acetylation of histones H3/H4, p53 and up-regulation of Bax [85].

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 [86].

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. [87] This is in agreement with a previous report showing that coexpression of HDAC1 reduced the activation potential of p53 on Bax promoter. [88] 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.

Since acetylation of p53 by p300 greatly enhances its DNA-binding ability, and hence the transactivation activity [89], 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. 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 [90].

The present study showed insignificant change in PARP activity between normal control group and butyrate treated group. Although the present study showed significant change in PARP activity between PD group and the two PD groups treated with sodium butyrate, and these results can be explained in the following paragraphs.

PQ neurotoxicity results in clinical, neurophysiologic, and biochemical parkinsonism. Animal models of PQ-induced parkinsonism have provided insight into mechanisms of neuronal death and spurred developments of pharmacologic treatments for PD. Reflective of mechanisms involving oxidative stress in the pathogenesis of PD, PQ toxicity leads to production of reactive oxygen species. [91] PQ enters the brain and is converted to the

complex I inhibitor. Complex I inhibition results in overproduction of superoxide anion (O_2^-), and mouse models of PQ-induced parkinsonism demonstrate a role for O_2^- in neuronal death. [92] Nitric oxide (NO) produced from neurons or glia, vianNOS and iNOS, respectively, are also instrumental in PQ-induced dopaminergic death. Peroxynitrite, a potent free radical capable of nitration and DNA damage is formed from the combination of NO and O2– and may mediate neuronal death [92].

Consistent with peroxynitrite formation, the brains of mice receiving PQ demonstrate evidence of nitration and DNA damage. PQ induced neuronal death follows DNA damage as suggested by activation of poly(ADP-ribose) polymerase (PARP-1) [93]. PARP-1 utilizes nuclear NAD to poly(ADP-ribosyl)ate a number of DNA binding proteins including auto modification of PARP-1. Chains of approximately 200 units of poly(ADP-ribose) polymer are covalently attached to acceptor proteins. In the presence of sufficient DNA breaks, PARP-1 consumes considerable NAD and hence ATP (from which NAD is derived in the nucleus). One target of PARP poly(ADP-ribosyl)ation is the tumor suppression protein p53, a nuclear protein that can direct cell death by transactivation of other genes [94].

PQ-induced dopaminergic death is dependent upon generation of free radical species and DNA damage. In response to DNA damage following PQ neurotoxicity, the nuclear enzyme PARP-1 is activated. DNA damage can also serve as a signal to activate p53, a response that inhibits the replication of cells with damaged DNA. P53 can also trans activate other proteins to induce apoptosis in non-neural lines. P53 levels are significantly increased following a toxic dose of PQ. This high level of poly(ADP-ribosyl)ation of p53 by PARP-1 can serve to stabilize p53 from degradation. Stabilization of p53 following DNA damage presumably serves as a step toward cellular apoptosis [95,96].

In addition to stabilizing p53, PARP-1 also appears to influence p53 activity following PQ intoxication. At times of heavy poly(ADP-ribosyl)ation, p53 fails to bind to p53 consensus sequences efficiently. Accompanying the attenuation of DNA binding is a reduction in the levels of the p53-dependent gene MDM-2. Two potential mechanisms whereby PARP may block p53 from binding to DNA include direct post-translational modification of p53 by PAR polymer, which would interfere with the p53 DNA binding domain, or indirectly by charge interference. A major function of PARP-1 is to facilitate double-strand DNA separation by placing many negatively charged PAR polymers on nuclear proteins allowing for enhanced repair of single-strand DNA nicks or breaks. A similar scenario likely prevents p53 transactivation as heavily poly(ADP-ribosyl)ated p53 is repelled from binding DNA. Consistent with this notion, p53 is poly (ADP-riboysl)ated on distinct domains that do not contain the DNA binding motif of p53 [97,98].

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