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Neuroprotective Role of Vitamin B_3 in Experimentally Induced Oxidative Stress

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Abstract Paraquat is a widely used herbicide. The main mechanism underlying PQ toxicity is oxidative stress. Niacin (nicotinic acid) a precursor for NAD+ It has also been reported to possess oxygen radical scavenging activity. The enzyme PARP-1 is activated by DNA strand breaks, using NAD+ as a substrate. Thus, the present study aimed to assess the magnitude of oxidative DNA damage and the role of PARP and the advantages of modulating its activity by niacin supplementation in experimentally induced oxidative stress by PQ. 50 male albino rats were equally divided into five groups; the first and second groups were the control and PQ treated groups respectively while the 3rd group was nicotinic acid treated group; the 4th and 5th groups were co- and post treated PQ treated rats with nicotinic acid respectively. Serum 8-hydroxy-2'-deoxyguanosine and brain MDA levels in PQ treated group showed a significant increase when compared with control group, while levels of PARP activity and TAC in PQ treated group showed a significant decrease when compared with control group. A significant increase of PARP activity & TAC and a significant decrease in serum 8-hydroxy-2'-deoxyguanosine&MDA after nicotinic acid injection when compared with control group was observed. Post-treatment with nicotinic acid improved the biochemical and histopathological alterations in brain treated with nicotinic acid, while co-treatment with nicotinic acid protected against ROS production.

Keywords: *Paraquat, Nicotinic acid, Oxidative stress, PARP-1 activity, 8-OHdG, MDA, TAC*

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1. Introduction

Paraquat (PQ) (1,1′-dimethyl-4,4′-bipyridium dichloride) is a widely used herbicide. Epidemiological data shows that paraquat poisoning is quite common, accidental or intentional [1]. In addition to the reported toxicity to the lungs and kidneys, it has toxic effects in the central nervous system as well [2]. Following systemic administration in rats, paraquat penetrates the blood–brain barrier causing neurotoxic effects and brain damage [3].

The main mechanism underlying PQ toxicity is oxidative stress due to overproduction of ROS [4]. During redox cycling, PQ undergoes an enzymatic one-electron reduction, forming radical cations. Under aerobic conditions, these radicals react rapidly with molecular oxygen, forming superoxide anion and H_2O_2 and, in the presence of redox-active transition metals, highly toxic hydroxyl radicals. These ROS can cause oxidative stress leading to damage to lipids, proteins, and nucleic acids [5].

Although ROS can damage a variety of cellular components, DNA is a critical target because it can lead to base modifications, abasic sites and double strand breaks, all of which can alter the information content of cells [6].

Poly (ADP-ribose) polymerase (PARP-1; EC 2.4.2.30) is a 116 kDa nuclear protein [7]. It is a zinc-finger DNA-binding enzyme [8] which is important in DNA damage responses, including repair, maintenance of genomic stability, signaling following stress responses that influences apoptosis, telomere function, transcription regulation, and numerous other cellular functions [9]. It detects and signals DNA strand breaks generated either directly during base excision repair, or indirectly by genotoxic agents such as oxygen radicals [10]. PARP-1 is differentially processed in apoptosis and necrosis, and therefore its activity can potentially be used as a means of distinguishing these two forms of cell death in response to DNA damage [10,11,12].

The enzyme PARP-1 is activated by DNA strand breaks, and catalyzes the formation of extensive linear and branched polymers of poly(ADP-ribose) attached to a protein acceptor using NAD^+ as a substrate ([13]). As a consequence of PARP-1 activation, cellular NAD+ is consumed. Over activation of PARP-1 after genotoxic stress leads to rapid NAD+ and ATP depletion and ultimately to cell death [10,11,14,15].

Niacin (vitamin B_3) is a water-soluble vitamin that occurs in two forms: nicotinic acid and nicotinamide. Both nicotinic acid and nicotinamide are dietary precursors for

the synthesis of nicotinamide adenine dinucleotide, NAD (H), and related coenzymes, NADP(H). In addition to its role as a cofactor in redox reactions and as a regulator of the redox state (NAD^+/NADH), NAD^+ functions as substrate for numerous classes of ADP-ribosyltransferases involved in cellular processes including transcription, calcium homeostasis, DNA repair, cell death, neoplastic transformation. NAD^+ is a substrate for the nuclear enzymes poly-(ADP-ribose) polymerases (PARPs) [9]. In addition to being niacin a precursor for NAD+ It has also been reported to possess oxygen radical scavenging activity [16].

Thus, the present study was aimed to assess the magnitude of oxidative DNA damage and the role of PARP and the advantages of modulating its activity by niacin supplementation in experimentally induced oxidative stress by paraquate. The markers that were chosen to detect oxidative stress; comprised serum 8-hydroxy-2'-deoxyguanosine as maker for oxidative DNA damage, tissue MDA level as marker for lipid peroxidation mediated by oxidative stress, TAC as a marker of total antioxidant compounds and PARP activity as a marker for activity of enzyme poly ADP polymerase.

2. Materials and Methods

2.1. Animals and Drug Treatment

50 male Albino rats (150–170) g. housed in an animal room maintained under constant temperature (23–25°C) with a 12: 12 light: dark cycle. Food and water were available ad libitum. Rats were randomly divided into 5 groups each group comprising 10 rats.

A control group; paraquat induced oxidative stress group; nicotinic acid treated group; nicotinic acid Co-treated group and nicotinic acid Post-treated group. The rats in paraquat treated group were treated with 20% PQ (10 mg/kg) body weight ([17]; Sigma–Aldrich, St. Louis, MO) in normal saline by intraperitoneal (i.p.) injection once weekly for 6 consecutive weeks, nicotinic acid treated group received i.p. injection of nicotinic acid in a dose of 100 mg/kg body weight [18]; Sigma–Aldrich, St. Louis, MO and dissolved in saline in a total volume of 1.0 ml per day for 6 weeks. Nicotinic acid Co-treated group, this group received i.p. injections of PQ (10 mg/kg) concomitent with nicotinic acid (100 mg/kg) for a total of 6 weeks. Nicotinic acid Post-treated group, rats received i.p. injections of 20% Paraquat for 6 weeks and thereafter treated with nicotinic acid for 6 weeks.

2.2. Neurochemical and Histological Experiments

The rats were killed by decapitation. Brains were quickly removed and divided in two parts; one piece from each specimen was fixed in 10% neutral buffer formalin, for histopathological study. The otherremaining pieces were washed and stored at – 80 C° till used for preparation of tissue homogenates, nuclear and DNA extracts. Blood samples were taken into dry sterile centrifuge tubes. Sera were separated and stored in aliquots at -80 °C till used.

2.3. DNA Damage - 8-OHdG

DNA damage was determined in serum samples using the 8-hydroxy-2-deoxy-guanosine (8-OHdG) EISA kit supplied by ENZO life sciences (USA). The absorbance was measured using ELISA reader at a wavelength of 450 nm [19].

2.4. Preparation of Nuclear Extractions

For estimation of total protein content & PARP activity by using Membrane, Nuclear & Cytoplasmic Protein Extraction kit supplied by BIO BASIC Inc. Canada. The addition of (cytoplasmic protein extraction) solution to the sample provides hypotonic condition that breaks cell membrane and releases proteins followed by centrifugation for collection of the nuclei. Then, the nuclear proteins were extract by adding nuclear protein extraction solution followed by centrifugation [20].

2.5. PARP-1 Activity

PARP-1 activity was assessed using (Universal Colorimetric PARP assay kit, Trevigen Inc, Gaithersburg, MD, USA). The assay measures the incorporation of biotinylatedpoly (ADP-ribose) onto histone proteins in cell homogenates according to manufacturer instructions. The absorbance was measured using ELISA reader at a wavelength of 450 nm [21].

2.6. **Estimation of protein content** in Nuclear Extract of Brain Tissue According to Lowry Method [22].

2.7. MDA

Malondialdehyde (MDA) was determined in brain tissue by spectrophotometric method at 534 nm using commercial kit supplied by Biodiagnostic, Egypt. Tissues were homogenised in Potassium phosphate buffer 50 mM (pH 7.4) [23].

2.8. Total Antioxidant Capacity

Total antioxidants were determined in brain tissue by spectrophotometric method at 505 nm using commercial kit supplied by Biodiagnostic, Egypt. Tissues were homogenised in Phosphate buffer saline 5 mM (pH 7.4) [24].

3. Results

Table 1 comparison of studied parameters among all studied groups. Table 1 indicates that a significant decrease (P <0.01**) in body weight gain in PQ group (G_2) when compared with all other groups. The present data showed that there was a significant increase (P<0.05*) in the serum level of 8-OHdG of PQ treated group (G_2) when compared with normal control group (G_1) and all the other groups. A insignificant (P >0.05) difference between the other groups when compared to each other. A significant decrease in the serum level of 8-OHdG in treated group with PQ and nicotinic acid (G_4&G_5) when compared with PQ treated group (G_1), but still there was insignificant decrease in the serum level of 8-OHdG in (G_4) when compared with(G_5). A significant decrease in PARP

activity of PQ treated group (G_2) when compared to normal control group (G_1) and all other groups. However, there was significant increase in PARP activity of (G_5) when compared to other groups. Also, insignificant difference in PARP activity between (G_4) and (G_1). A significant increase in brain MDA level of (G_2) when compared to control group (G_1) and all other groups. And there were significant increase in brain MDA level of (G_4) and (G_5) when compared to brain MDA level of (G_1) and (G_3). Also we found that there was a significant difference in brain MDA level between (G_4) and (G_5). A significant decrease in brain total antioxidant capacity (TAC) level of PQ treated group (G_2) when compared to control group (G_1) and the other groups. And there was a significant decrease in brain TAC level of (G_4) and (G_5) when compared to (G_1) and (G_3). A significant difference in brain TAC level between (G_4) and (G_5).

Table 1. Changes in weight gain of rats (gm), serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), PARP activity (Unit/mg protein) of nuclear extract, brain malondialdehyde (MDA) and brain total antioxidant capacity (TAC) levels in different groups under study

	G_1	G_2	G_3	G_4	G_5	ANOVA		Tukey's test
						F	P	
Body weight gain (gm)	0.39 ± 0.029	0.29 ± 0.018	0.38 ± 0.028	0.39 ± 0.025	0.42± 0.031			$P(G_2$ vs $G_1,G_3,G_4)$ <0.05* $P(G_2$ vs $G_5)$ <0.01**
Serum 8-OhdG (ng/ml)	2.13± 0.217	7.08 ± 0.69	2.50± 0.19	2.60 ± 0.08	3.40 ±0.517	17.364	< 0.0001*	$P(G_2$ vs G_1, G_3,G_4, $G_5)$ <0.001***
PARP activity (Unit / mg protein)	0.20 ±0.021	0.06± 0.008	0.16 ±0.009	0.17± 0.011	0.59± 0.033	84.941	< 0.0001*	$P(G_5$ vs G_1, G_2, G_3, G_4) <0.001*** $P(G_2$ vs $G_1,G_4)$ <0.01** $P(G_2$ vs $G_3)$ <0.05*
Brain MDA (nmol /g tissue)	752.6± 20.2	1913 ± 73.1	721 ± 22.7	1058± 83.8	1380± 39.3	82.06	<0.0001*	$P(G_2$ vs G_1, G_3,G_4, $G_5)$ <0.001*** $P(G_1$ vs $G_4)$ <0.05* $P(G_1$ vs $G_5)$ <0.001*** $P(G_4$ vs G_3, $G_5)$ <0.05* $P(G_3$ vs $G_5)$ <0.001***
Brain TAC (mmol/g tissue)	930.8 ± 39.2	353.7± 22.6	984.8 ± 17.9	809.2 ± 4.28	698 ± 15.2	123.44	< 0.0001*	$P(G_2$ vs G_1,G_3,G_4, $G_5)$ <0.001*** $P(G_1$ vs $G_4)$ <0.05* $P(G_1$ vs $G_5)$ <0.001*** $P(G_4$ vs $G_3,G_5)$ <0.05* $P(G_3$ vs $G_5)$ <0.001***

Data are expressed as mean ± S.E.M of 10 observations. Where control group (G_1), PQ group (G_2), Nicotinic acid group (G_3), Nicotinic acidco-treated group(G_4) and Nicotinic acid post-treated group (G_5).

4. Discussion

The main mechanism underlying PQ toxicity is oxidative stress due to overproduction of ROS [4]. Although ROS can damage a variety of cellular components, DNA is a critical target because it can lead to base modifications which can alter the information content of cells [6]. 8-OHdG is the most reliable and abundant marker of DNA damage because it is a sensitive index as it reflects extremely low levels of oxidative damage [25].

The present data showed that there was a significant increase in the serum level of 8-OHdG of group 2 when compared with control group and all the other groups. The increment in the serum level of 8-OHdG in the current work can be explained on the base that when PQ is absorbed into the body, ROS is produced to attack DNA bases and in turn to form a variety of modified bases such as 8-hydroxyguanine, 8-hydroxyl adenine, cytosine glycol, and thymine glycol. Guanine molecules contain high orbital energy, resulting in accelerating generation of 8-OHdG [26].

The decrement of 8-OHdG level of group 4 and group 5 when compared to group 2, indicating that niacin reduces the induction of ROS production. This result confirms findings of limited previous studies of Chapman et al. [27]; Ganji et al. [28]; Ganji et al. [29]. PARP activity is an extremely sensitive indicator of DNA damage.The results showed that there was a significant decrease in PARP activity of group II when compared to group I and all other groups and there was significant increase in PARP activity of group V when compared to all other groups.

So, it can be postulated that oxidative stress induces chronic and systemic PARP-1 activation and increase NAD^+ turnover. Boulares et al. [30] reported that PARP-1 over activation followed PARP cleavage in apoptosis has been investigated in human osteosarcoma cells. During apoptosis, caspase-7 and caspase-3 cleave PARP-1 into two fragments. These proteases recognize the nuclear localization signal of PARP-1, and cleavage at this site separates the DNA binding domain from the catalytic domain, resulting in the inactivation of the enzyme [31]. This inactivation of PARP has been proposed to prevent depletion of NAD (a PARP substrate) and ATP, which are thought to be required for later events in apoptosis [30].

Surprisingly, the group that received nicotinic acid after 6 weeks as in group 5 showed marked increase in PARP activity this can be explained by PARP over activation which is not followed by cleavage and apoptosis as the nicotinic acid ; the dietary precursor for NAD^+, which is required for the activity of the enzyme PARP-1; decreased the marked oxidative stress present and the vast majority of dying cells arrested the apoptotic process and recovered when the inducer was washed away [32].

In the present study a significant increase in brain MDA levels, as a marker of oxidative stress, in group 2 compared with (group I) has been detected which suggested a severe oxidative stress state. The highest level of MDA in group of rat given PQ is consistent with the findings from several studies that exposure of animals to PQ resulted in significant increase in lipid peroxidation [33].

The decrease in brain TAC level may be due to redox cycling reaction: (i) the generation of the superoxide anion which can lead to the formation of more toxic reactive oxygen species, such as hydrogen peroxide and hydroxyl radical; (ii) the oxidation of the cellular NADPH, the major source of reducing equivalents for the intracellular reduction of paraquat, which results in the disruption of important NADPH-requiring biochemical processes, and (iii) lipid peroxidation which results in the oxidative degeneration of cellular polyunsaturated fatty acids [34].

The increase in brain TAC of group 4 and group 5 when compared with group 2. This result confirms findings of limited previous studies [29] that demonstrated niacin significantly increased GSH levels by 98% and GSH/GSSG ratio compared to control in cultured human aortic endothelial cells (HAEC) ring the oxidative stress and so lowering the oxidative stress induced by PQ. The improvement of markers of oxidative stress of group 4 could be explained that nicotinic acid and paraquat competed for the same binding site on complex I. Nicotinamide, which has a pyridine ring similar to the nicotinic acid, might have an inhibitory effect on paraquat toxicity [35].

Excess nicotinamide administration might cause a conformational change of the ferricyanidereaction site on complex I. These results imply that nicotinamide has an inhibitory effect against paraquat toxicity. Nicotinamide, paraquat, and ferricyanide may react at overlapping sites, probably near the 30 kDa subunit, on complex I. These sites should be important to establish for effective treatment against paraquat poisoning [36].

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