www.ajlifesciences.com / ISSN: 2663-1040 *AJ Life Sci. 2026, 9 (1): 12-18* **Research Article**

Abasyn Journal of Life Sciences

Open Access

DOI: 10.5281/zenodo.1825414.8

Examination of the Neuroinflammatory Effects of Oral Polio Vaccine in Mice Neonates

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Abstract

In the present study the neurotoxicity of polio vaccine was checked by orally administering the polio vaccine to day seven 7 mice neonates. Different doses (in drops form) were administered to the mice neonates. The doses were low, moderate and high as per number of drops such as 1, 2 and 4 respectively. Postnatal day 7 (PND-7) mice pups were divided into four groups including i. Control (C), ii. Low Dose (LD), iii. Moderate Dose (MD), iv. High Dose (HD). Due to its importance in research this model is compatible with 3rd trimester of pregnancy in the human beings.All the pups were sacrificed after 4 hours of the polio vaccine administration. The brain was collected very carefully. The brains from all the animals were subjected to Western blot technique. The results indicated there was no significant difference among the expression of proteins involved in the neuro inflammation and neurodegeneration in the first three groups like C, LD and MD. In contrast to this the high dose induced significant neurotoxicity in the mice brain and the expression levels of neuro inflammatory and neurodegeneration proteins were significantly high as compared to the control and other treated groups. These proteins include phosphorylated JNK (C-Jun Terminal Kinase), NF-kB (Nuclear factor kappa B) and PARP-1 (Poly (ADP-ribose) polymerase 1). In summary this study shows that among the four groups LD and MD are nontoxic while the HD is neurotoxic to the immature mice brain, hence it is a lethal dose in case compared to the human beings.

Keywords: Oral Polio Vaccine, Neuroinflammation, Mice Neonates, p-JNK, NF-κB, PARP-1, Neurotoxicity.

Article Info:

Received:
December 24, 2025
Received Revised:
January 07, 2026
Accepted:
January 07, 2026
Available online:
January 15, 2026

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1. INTRODUCTION

Michael Underwood, a physician in 1789 was the first who demonstrated the clinical description wherein, polio was called as "poor condition of the lower body organs''[1,2]. This disease-commonly known as polio, is a pathogenic disease due to the poliovirus. Its other name is Infantile Paralysis. There three serotypes of polio virus that cause poliomyelitis: PV1, PV2 and PV3[3,4,5]. The causative agent of polio, also known as poliomyelitis is Poliovirus, which was first identified in 1908. It is an ancient disease as described in ancient art, existed for thousands of years. This turned out to be one of the most concerning childhood illnesses in the 20th century[6].

The spinal cord is the most commonly involved, resulting in the characteristic clinical presentation of clinical paralysis. Among all cases of polio infections, the nervous system is rarely infected, it's about 1%. Acute flaccid paralysis is a condition about one to five in 1000 cases, wherein the muscles begin to become

flaccid, fragile and unresponsive due to weakening with a complete paralysis. Paralytic polio is classified on the basis of site of paralysis. Depending on the site of paralysis, it is categorized into three distinct types as follow. Spinal polio – it is the most prevalent form of polio and from 1969-1979, about 79% of the cases were diagnosed as Spinal polio. The leading clinical type is still polio with spinal manifestations[7,8,9]. This is characterized by a predominance of asymmetric paralysis that frequently affects the legs. The second type of polio, Bulbar polio - only accounts for 2% of cases and results in the weakness of the muscles supplied by cranial nerves. The third variety is Bulbo-spinal polio - which constitutes 19% of cases and is characterized by paralysis involving both the bulbar and spinal regions

2. MATERIALS AND METHODS

2.1 Experimental Design

PND-7 mice pups were randomly divided into four groups (n=3 per group):

i) Control (C): no vaccine administered

ii) Low Dose (LD): 1 drop OPV

iii) Moderate Dose (MD): 2 drops OPV

iv) High Dose (HD): 4 drops OPV

After 4 hours of vaccine administration, pups were sacrificed, and their brains were dissected and preserved at −80°C.

2.2 Protein Extraction and Quantification

Brains were homogenized in tissue-protein extraction reagent (pH 7.6) using a homogenizer. The samples gothrough a centrifugation process at 14,000 rpm for 25 minutes keeping the temperature at 3°C. The concentration of proteins was evaluated by UV spectrophotometry employing the Bio-Rad protein assay. Average optical densities (OD) were used to calculate protein concentrations and standardize loading amounts for Western blotting [10,11,12].

2.3 Western Blot Analysis

Equal amounts of protein (30 µg) were subjected to 15% SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 5% skim milk for one hour and incubated overnight with primary antibodies against p-JNK, NF-κB, and PARP-1 at 4°C. Following the washing process, the membranes were treated with secondary antibodies conjugated to HRP and then the signals were detected through the method of enhanced chemiluminescence (ECL) [13]. The intensities of the signals were measured in order to determine the amounts of the proteins present[14,15].

3 RESULTS AND DISCUSSIONS

3.1 Oral Polio Vaccine in a Dose Dependent Manner Induced Phospho-JNK Activation in Mice Neonates

In the current study we have used polio vaccine in a dose dependent manner (1, 2 and 4 drops) given orally to the postnatal day 7 mice pups. As this model is very important in research and is compatible with 3rd trimester of the human beings. All the animals except the control mice were given polio vaccine orally i.e. 1, 2 and 4 drops respectively. All the pups were sacrificed after 4 hours of the polio vaccine administration. The brain was collected very carefully. The brains from all the animals were subjected to Western blot

technique. The western blot results indicated the two doses both low and mild have no significant difference as shown in the figure 3.1. Interestingly the high dose shown some significant effect by activating phosphor-JNK as given in the figure 3.1

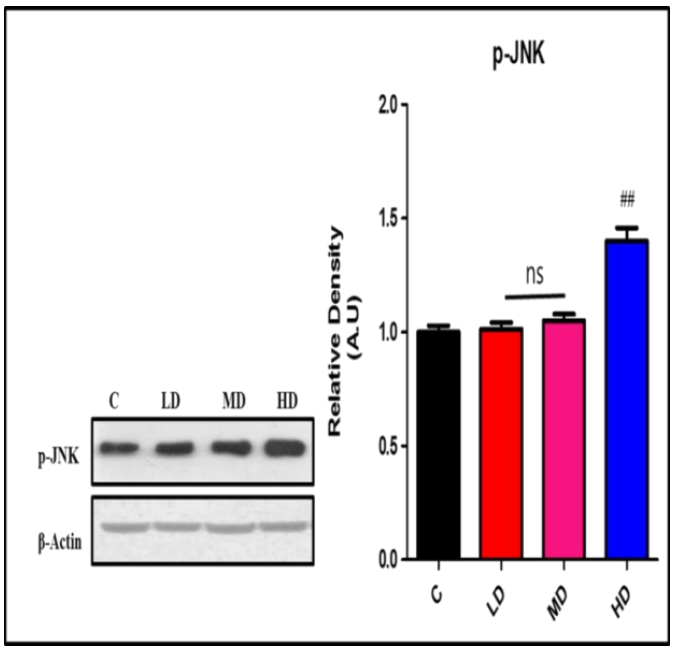

Figure. 3.1: The Western blot of P-JNK and its histogram respectively in 7 day mice neonates.

A study reported[16] an animal neurotoxicity assay for vaccinia-based smallpox vaccines and initially characterized, where Dryvax virus was used as reference (vaccine) strain. They used neuro adapted Western Reserve (WR) strain as neurotoxic positive control. In mice that were inoculated neonatally, the WR strain caused mortality that was significantly higher and of more rapid onset than that of the Dryvax vaccine reference. Expression of virus antigen in neural cells and infectious virus reproduction in the brain also varied between the strains. Moreover, the presence of high titer virus antibodies was found to be associated with the clearance of the virus from the brain. They validated further that this assay, which included a vaccine reference standard (licensed) and a positive control strain, might present a critical pre-clinical neurotoxicity data on other smallpox vaccine strains.

3.2 Oral Polio Vaccine in a Dose Dependent Manner Induced NF-Kb Activation in Mice Neonates

In the current study, we have used polio vaccine in a dose dependent manner (1, 2 and 4 drops) given orally to the postnatal day 7 mice pups. As this model is very important in research and is compatible with 3rd trimester of the human beings. All the animals except the control mice were given polio vaccine orally i.e. 1, 2 and 4 drops respectively. All the pups were sacrificed after 4 hours of the polio vaccine administration. The brain was collected very carefully. The brains from all the animals were subjected to Western blot technique. The western blot results indicate that the two doses both low and moderate have no significant difference as shown in the figure 3.2. Interestingly the high dose shown some significant effect by activating NF-kB as given in the figure-3.2.

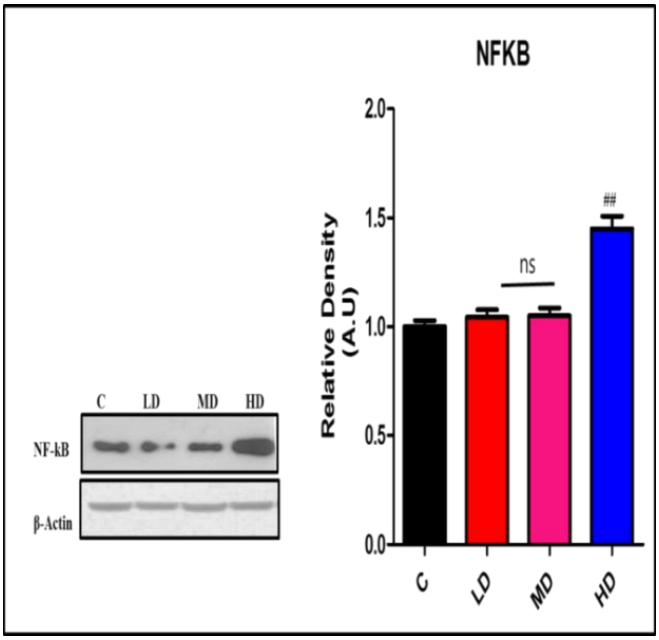

Figure 3.2: The Western blot of NF-kB and its histogram respectively in 7 day mice neonates.

NF-κB is a key transcription factor regulating inflammatory and immune responses within the central nervous system. One study described[17] NF-κB as a crucial mediator of neuroinflammation, linking immune activation to neuronal dysfunction and increased vulnerability to injury in the developing brain. More recently, other studies reported[18] that dysregulated NF-κB signaling in the immature central nervous system promotes microglial activation and excessive production of pro-inflammatory cytokines, ultimately disrupting neuronal homeostasis. The selective activation of NF-κB observed in the high-dose oral polio vaccine group in the present study is consistent with these findings, suggesting that excessive vaccine exposure may exceed the regulatory capacity of neonatal immune responses and initiate inflammatory signaling cascades, while standard low and moderate doses remain biologically safe.

In a very similar manner, LeaRozenstein-Tsalkovich group [19] described the vaccination with phos-tau peptides under a CNS pro-inflammatory condition. NFT and WT-mice were given the phos-tau peptides in a total of 7 injections for adult mice and 4 for aged ones. Incidence of paralyzed disease among the adult NFT-mice showing phos-tau-immunization increased with the number of injections, and 26.7% was reached in the end of the treatment. WT-mice were even more likely to suffer from neuroinflammation, as consequence of phos-tau immunization with 75% of the immunized mice suffering from it. Older mice had lesser likelihood of showing such neuroinflammatory symptoms. The presence of anti-phos-tau antibodies in the mice's serum that received the immunization was partially correlated with the neuroinflammation in WT-mice.

3.3 Oral Polio Vaccine in a Dose Dependent Manner Induced DNA Damage in Mice Neonates

In the current study, we have used polio vaccine in a dose dependent manner (1, 2 and 4 drops) given orally to the postnatal day 7 mice pups. As this model is very important in research and is compatible with 3rd trimester of the human beings. All the animals except the control mice were given polio vaccine orally i.e. 1, 2 and 4 drops respectively. All the pups were sacrificed after 4 hours of the polio vaccine administration. The brain was collected very carefully. The brains from all the animals were subjected to Western blot technique. The western blot results indicated that the two doses both low and mild have no significant difference as shown in the figure 3.3. Interestingly the high dose showed some significant effect by activating PARP-1 as given in the figure 3.3.

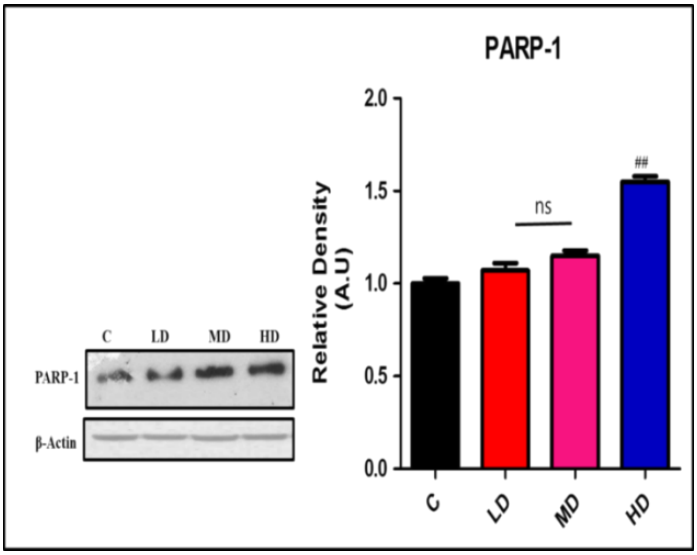

Figure 3.3: The Western blot of PARP-1 and its histogram respectively in 7 day mice neonates.

The c-Jun N-terminal kinase pathway is a well-recognized stress-activated signaling cascade involved in neuronal injury, inflammation, and apoptosis. A study[20] reported that sustained activation of JNK plays a central role in neuronal stress responses and neuroinflammatory processes, particularly in immature neural tissue. Similarly, another study [21] emphasized that excessive JNK signaling contributes to neuronal apoptosis and neurodegeneration under pathological conditions. Supporting these observations, Li et al. [21] used dose-dependent neurotoxicity in neonatal mouse models exposed to vaccine strains, where higher antigenic loads resulted in increased neural damage and mortality. In agreement with these earlier reports, the present study shows that phospho-JNK activation occurs only at excessively high oral polio vaccine doses, indicating that elevated antigenic exposure may overwhelm endogenous protective mechanisms in the developing brain.

PARP-1 is widely recognized as a molecular marker of DNA damage and cellular stress, particularly under neuroinflammatory and neurodegenerative conditions. Some studies [22] reported that excessive activation of PARP-1 contributes to neuronal death by depleting intracellular NAD^+ and ATP levels, thereby promoting apoptotic and necrotic pathways. Similarly, previous studies [16] demonstrated that sustained overactivation of PARP-1 during inflammatory stress leads to irreversible neuronal injury. In line with these reports, the increased PARP-1 expression observed in the high-dose group of the present study suggests that excessive oral polio vaccine exposure may induce acute genomic and metabolic stress in the immature brain, whereas low and moderate doses do not reach a neurotoxic threshold.

In the same way,other reports[23] demonstrated if Rubella is infected during the first 12 weeks of pregnancy, there will be a 80% chance of having congenital abnormalities. However, if a woman gets reinfected in the early days of pregnancy, the risk is very small. Prenatal diagnosis would be a good option to determine the risk of the fetus. Congenital rubella is not a static condition, and sometimes it will be the case that the infant is born with no visible malformations. The diseases Rubella and congenital rubella are often detected through the finding of rubella-specific IgM; a diagnosis of congenital rubella in infants over 3 months of age may be hard to confirm. The rubella vaccines are typically given along with those for measles and mumps. Their administration has made it possible for some developed countries to wipe out rubella and congenital rubella entirely. Countries should ensure that susceptible women of child-bearing age and health care workers are offered a rubella-containing vaccine. Rubella vaccine is contraindicated during pregnancy, but if a pregnant woman is inadvertently vaccinated it is not an indication for termination or prenatal diagnosis.

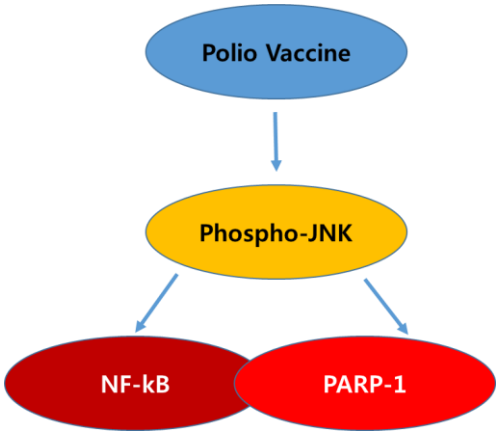

Figure 3.4: Oral Polio Vaccine in a dose dependent manner signaling pathway activation in mice neonates.

4. CONCLUSIONS AND RECOMMENDATIONS

Our study found oral intake of polio vaccine causes neuroinflammatory and neurotoxic effects in PND-7 mice neonates. Low doses of the vaccine comprising 1-2 drops did not show any adverse effects on neuroinflammatory markers or DNA damage-related proteins, showing its safety in the brain development. On other hand, high dose of vaccine comprising four drops caused significant activation of concerned signaling pathways. These findings indicate the composition of safe vaccine dose level which may be considered during vaccine development and administration.

NOVELTY STATEMENT

This study reported information about vaccine, its safe and harmful dosage and its harmful effect when high dose administered orally.

AUTHOR'S CONTRIBUTION

Sajid salam did the ewxperimental work, Hafsa and Hammad prepared the manuscript, Saqib and Imran did the analysis and Muhammad Shakeel did proof reading, guidance and submission to Journal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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