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LETTER TO THE EDITOR

Paraquat Toxicity on Substantia Nigra: Pioneering Insights from an Autopsy Based Pilot Study

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Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) is one of the widely used herbicides in Indian agriculture. This compound is at the centre of polemics, involving debates on its effects on sustainable agriculture, potential health risks, and unintended applications. Paraquat (PQ) also gained ill repute globally due to the controversy surrounding its association with Parkinson's disease [1]. Furthermore, our country witnessed a rise in paraquat-related deaths attributed to its misuse for suicide, prompting a consistent call for proper regulation and a potential ban [2]. Paraquat exerts its toxicity through corrosion and the generation of Reactive Oxygen Species (ROS) causing cytotoxic effects that contribute to multiorgan failure. There is no specific antidote for paraquat (PQ) poisoning, and the lethal toxicity of this substance is responsible for a poor prognosis, even with minimal consumption.

Paraquat neurotoxicity studies in Wistar rats indicated changes, including the loss of dopaminergic neurons and astrocyte proliferation, with the degree of changes escalating proportionally with increased dosage [3]. However, human autopsy-based studies on this subject are limited. A thorough review of literature (light microscopy and electron microscopy) in cases of paraquat and diquat poisoning indicated a few neuropathological changes, encompassing findings such as brain oedema, haemorrhagic leukoencephalopathy, anoxic neuronal depletion, myelin destruction, astrocytic fibrous gliosis, and hypoxic purpuric staining in the basal ganglia etc. [4].

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In the context of limited availability of human studies on histopathological changes in the substantia nigra related to paraquat poisoning, a pilot project was initiated. A thorough case selection process was conducted, excluding deceased individuals with pre-existing neurodegenerative disorders or any neuropathology. This pilot study was conducted collaboratively between the Department of Forensic Medicine and the Department of Pathology at Andhra Medical College, Visakhapatnam, spanning a 6-month period from August 2023 to January 2024. We utilized formalin (immersion)-fixed brain samples obtained during forensic autopsies conducted at our institute. These specimens were immersed and fixed in 10 percent formalin for a duration of 3-6 weeks. It is crucial to note that all samples were derived from cadavers promptly preserved in cold storage at 4 degrees Celsius as early as possible following death, ensuring the preservation of the brains under standardized conditions to the extent possible.

The substantia nigra of fixed brains in paraquat poisoning cases (n=9, 5 Males, 4 Females) was subjected to histopathology, stained with Hematoxylin and Eosin, and compared with a control group (n=6, 5 Males, 1 Female) comprising autopsy cases with causes of death unrelated to paraquat poisoning. The details of the cases and controls are presented in Table 1. The diagnosis of poisoning was confirmed through postmortem chemical analysis of viscera in all the cases.

The examination of the substantia nigra in post-mortem brain specimens in the cases revealed distinctive neuropathological changes compared to controls. Specifically, the substantia nigra in paraquat-exposed brains exhibited a considerable reduction in neuronal density, accompanied by oedema and a decrease in neuromelanin pigment content. The neuropathological features were accentuated in long-term survivors, characterized by astrocytosis, rarefaction and inflammatory markers (Figures 1-7). Contrastingly, the substantia nigra of the control brains demonstrated a superior staining affinity, indicating a dense congregation of neurons without discernible rarefaction and normal neuromelanin pigment levels (Figures 7-11). These observations highlight a notable disparity between the substantia nigra of cases and controls, implicating the neurotoxicity of paraquat.

The current pilot study is a pioneering attempt in the domain of paraquat neurotoxicity. Nevertheless, our results are concordant with an MRI-based imaging study in acute paraquat poisoned patients which revealed CNS toxicity and lesions in multiple areas of brain, including the basal ganglia [5]. Indeed, studies that integrate clinical examination of patients with MRI imaging will yield fruitful results in understanding the acute toxicity of PQ on humans. By the same token, conducting serial follow-ups with PQ poisoning survivors will help us comprehend the subacute and chronic toxicity of this substance on the basal ganglia, shedding light on its potential association with Parkinson’s disease.

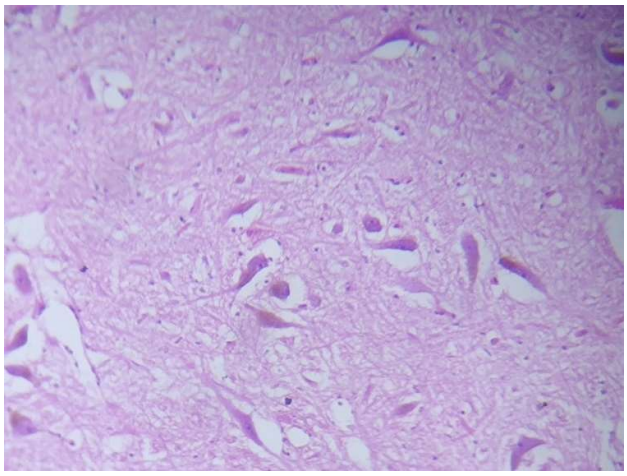
In conclusion, the present study indicates that paraquat crosses blood brain barrier and exerts its toxicity on brain in general and substantia nigra in particular. However, the limitations of the present study include its reliance on a limited sample size, and the absence of exact age-matched controls. Similarly, special stains were not employed for the examination of brain tissue. Future studies are needed to further validate the results of our study preferably with a larger sample size and more standardized sampling and processing

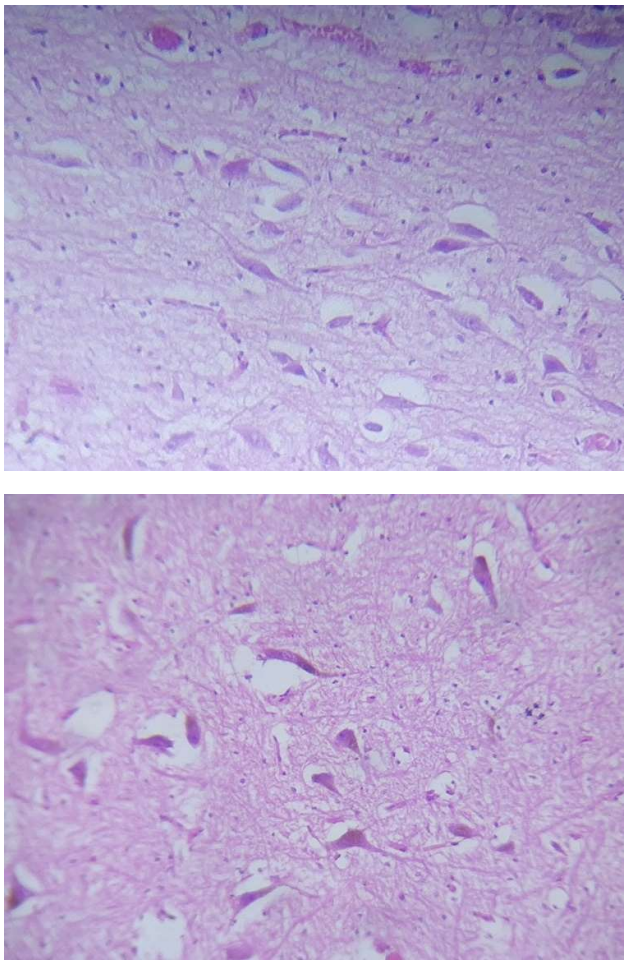
conditions to minimize the impact of postmortem artifacts. The significance of traditional autopsy and virtopsy in investigating toxicopathology is poised to become a pivotal subspecialty within the field of toxicology in the near future.

Table 1. Details of Cases and Controls in the Pilot Study

S.No	Case/Control (Age (in years) & Sex)	Time Since Death(in hours)	Time Since Consumption of Paraquat (in hours)	Cause of death
1	Case 1(22, F)	15	72	PARAQUAT POISONING
2	Case 2(31,F)	16	48	
3	Case 3(58,M)	15	29	
4	Case 4(20,F)	19	52	
5	Case 5(21,M)	10	168	
6	Case 6(20,M)	23	42	
7	Case 7(21,F)	18	264	
8	Case 8(29,M)	12	54	
9	Case 9(22,M)	16	25	
10	Control 1(32,M)	26	NOT APPLICABLE	Cut Throat Injury
11	Control 2(59,M)	24		Blunt Injury to the Chest
12	Control 3(29,M)	25		Olanzapine Poisoning
13	Control 4(53,M)	98		Coronary Artery Disease
14	Control 5(38,M)	24		Hanging
15	Control 6(6,F)	15		Thermal Burns

M: Male, F: Female





Figures 1-3. Photomicrograph H & E, High Power.
Cases 1-3, Substantia Nigra Showing a Decrease in Neuromelanin Pigmentation, Paraquat Poisoning.

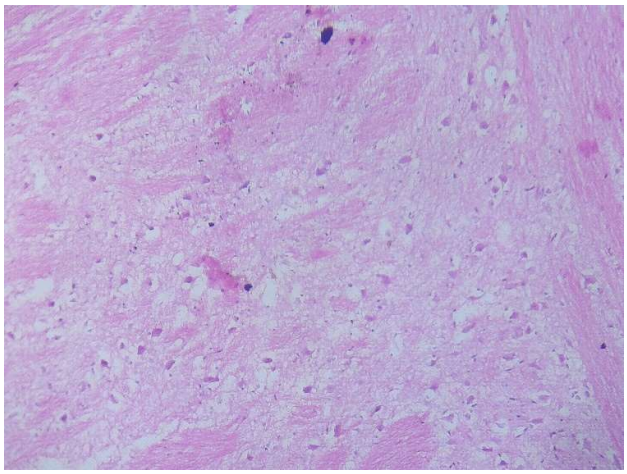


Figure 4. Photomicrograph H & E, Scanner View
Case 5, Substantia Nigra Showing A Decrease In Neuronal Density, Paraquat Poisoning.

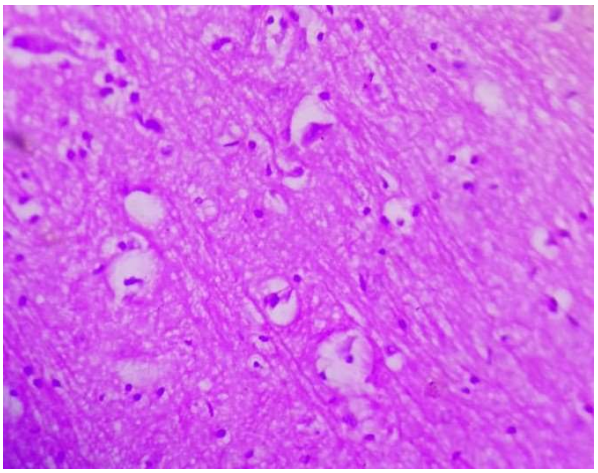


Figure 5. Photomicrograph H & E, Low Power.
Case 5, Substantia Nigra Showing A Decrease In Neuronal Density, Paraquat Poisoning.

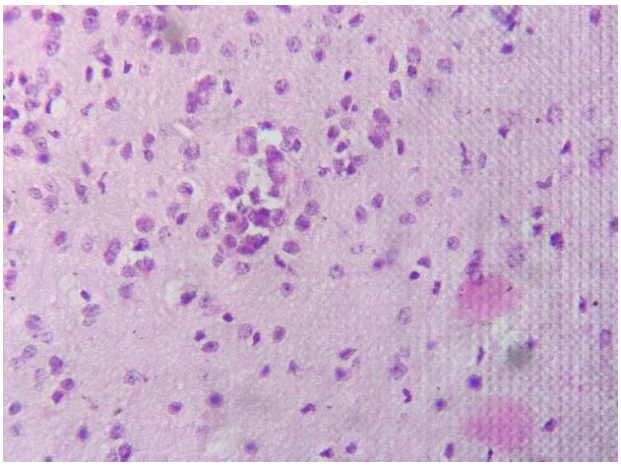


Figure 6. Photomicrograph H & E, High Power
Case 7, Substantia Nigra Showing Astrocytosis, Paraquat Poisoning.

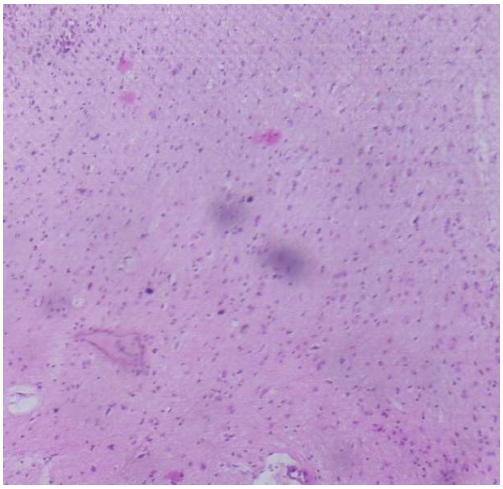
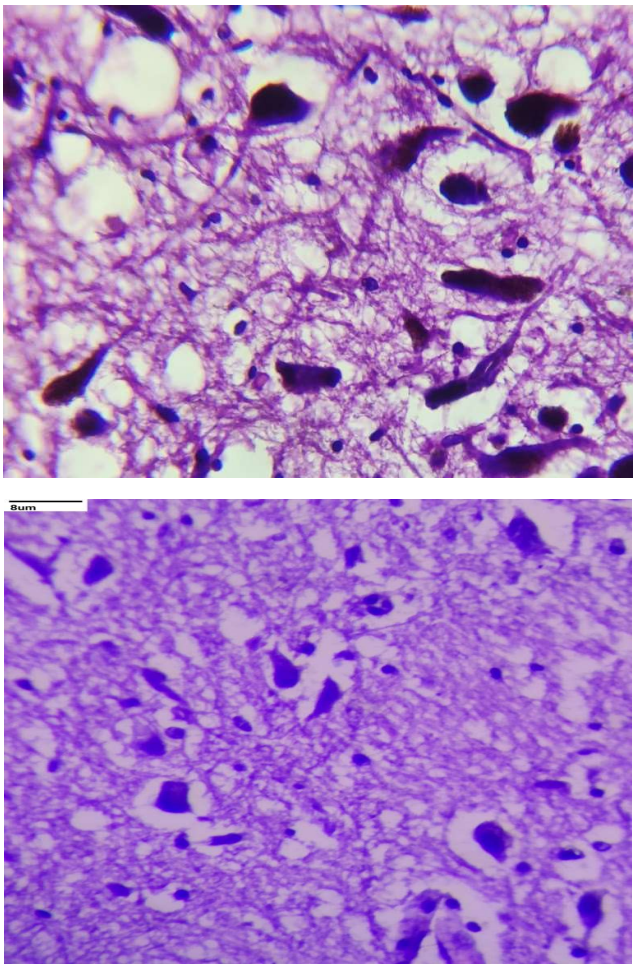


Figure 7. Photomicrograph H & E, Low Power
Case 7, Substantia Nigra Showing Astrocytosis, Paraquat Poisoning.



Figures 8-9. Photomicrograph H & E, High Power

Control 3, Showing Neuromelanin In Substantia Nigra (Normal Histology)

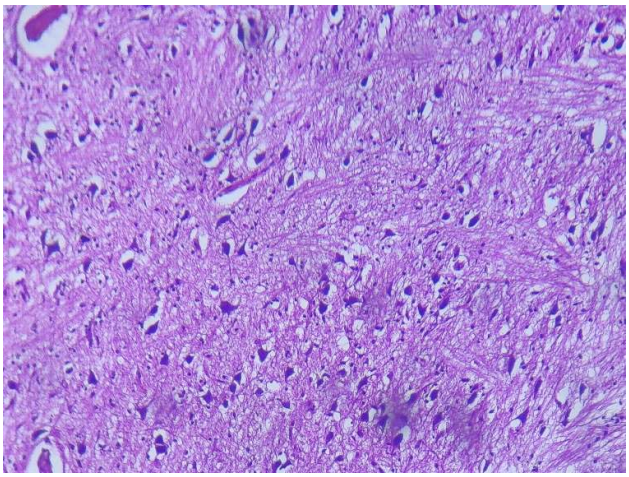


Figure 10. Photomicrograph H & E, Low Power

Control 5, Showing Normal Histology of Substantia Nigra

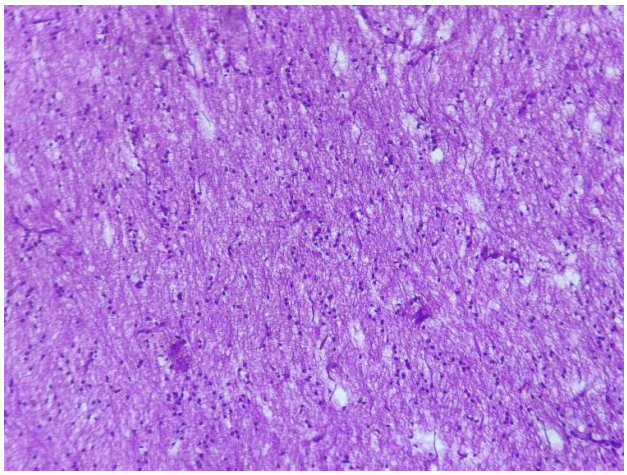


Figure 11. Photomicrograph H & E, Scanner View.

Control 2, Substantia Nigra Showing Normal Histology

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Ethical Issues

All ethical issue to be addressed by the authors

Conflicts of interest

The authors declares that they do not have conflict of interest.

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