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Perfluorocarbon (Oxycyte™) as Innovative Therapy post Spinal Cord Injury

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ABSTRACT

Spinal Cord Injury (SCI) is a life-altering event, which presently, cannot be reversed. Key components of the secondary injury cascade are an inadequate blood supply (ischemia) present at the injury site, leading to a decrease in oxygen delivery (hypoxia), and possibly neuronal cell death (apoptosis). However, a third generation Perfluorocarbon (Oxycyte™), at the appropriate dosage, can improve oxygenation of the injured tissue and overall motor behavior. To test this hypothesis, adult Long-Evans rats were divided into six experimental groups, given different doses of Saline or Oxycyte as treatment, with a focus on the 5 mL/kg Oxycyte group. After performing the initial surgery on the spinal cord, a weight-dropping device was used, to mimic SCI, and the respective treatment was given. Post operation, rats were subjected to scoring according to the BBB scale and inclined plane test on specific days after surgery, to determine improvement on a functional level. After completing functional tests, rats were euthanized for various lab tests, including histopathology and immunohistochemical analyses, to determine the key apoptotic related proteins. In summary, our results indicated that 5 mL/kg Oxycyte significantly improved motor function compared to 2mL/kg Oxycyte and the Saline control. However, more research of the optimal dose needs to be conducted.

INTRODUCTION

Spinal Cord Injury (SCI) is a traumatic event that has a severe impact on the quality of life of the individual (Bunge). Despite the progression of research and rehabilitation, no safe or effective treatments have been found to reverse the effects of the primary SCI (Cadotte). As a result of the primary injury, a drastic reduction of blood supply (ischemia) leading to a reduction of oxygen (hypoxia) is evident in the secondary injury and methods to prevent this drop are currently under investigation (Rowland). A promising contender, having a strong affinity for O₂, is usage of intravenous Perfluorocarbon therapy, specifically Oxycyte™ (Woitzik). The oxygen carrier, Oxycyte, at the appropriate dosage, can improve the recovery of motor function, preserve myelin and white matter, and reduce apoptotic neuronal cells (Yacoub). Through this research study, a significant improvement in motor function and neuronal preservation can be observed in adult Long-Evans rats with the usage of 5 mL/kg Oxycyte post Spinal Cord Injury, validated by functional and molecular tests.

MATERIALS AND METHODS

Adult Long-Evans rats were divided into six experimental groups, including a control, an injury with no treatment, rats receiving 2 mL/kg or 5 mL/kg Saline treatment, and rats receiving 2 mL/kg or 5 mL/kg Oxycyte treatment (Image 1). The control group had a sham surgery conducted, while the other five groups had a laminectomy performed at the T9 level of the spinal cord (Image 2). The five groups were subjected to the same injury by a NYU 10g weight-dropping device from the height of 25 mm at the T9 level (Image 3). The respective treatments were given once the external jugular vein was exposed (Image 4). Post spinal cord injury, all rats were monitored daily and subjected to being scored according to the Basso, Beattie, and Bresnahan (BBB) Locomotor scale and the incline plane test, to indicate their improvement on a functional level, day 1, 4, 7, 14, 21, 28, 35, and 42 post-operation. After the recovery-testing period, all groups of rats were euthanized, and lab tests, histopathology and immunohistochemical analyses, were conducted to determine the rehabilitation of the rats on a molecular level.



Image 1: Adult Long Evans rats

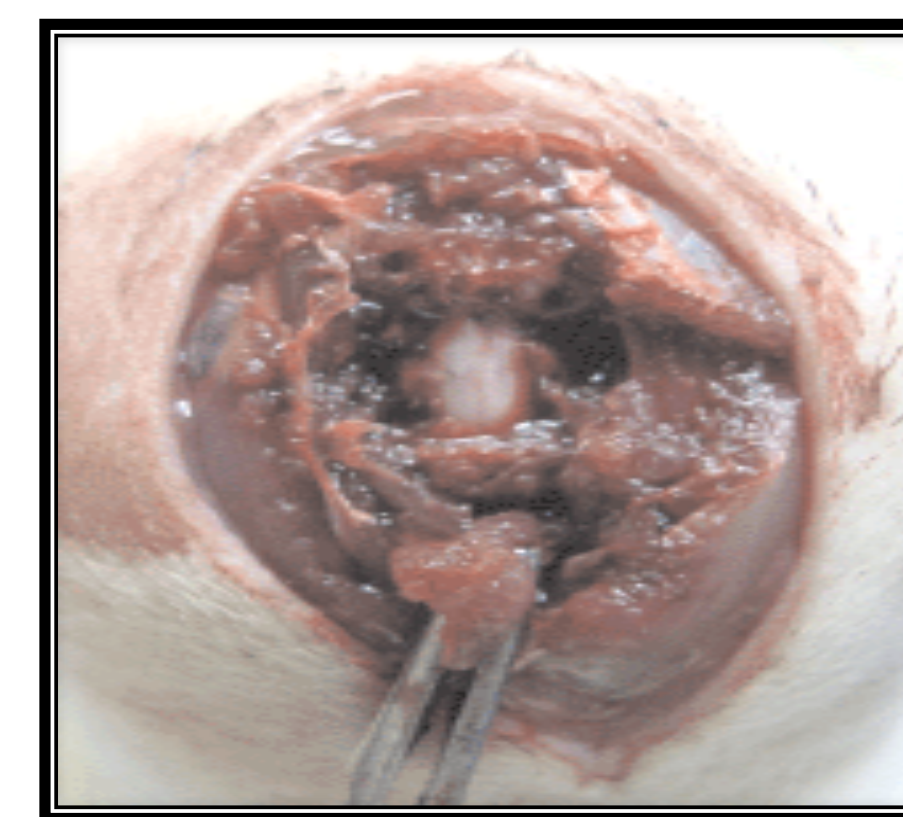


Image 2: Exposed Dura at T9 post Laminectomy



Image 3: NYU Impactor (Weight dropping device)



Image 4: Exposed Jugular Vein

RESULTS

Motor Function: Results from scoring according to Basso, Beattie, Bresnahan (BBB) Locomotor scale, indicated a statistical improvement in both doses of Oxycyte treatment compared to the Saline treatment, day 7, 14, 21, and 42 post SCI (Figure 1). Even though both Oxycyte doses showed strong recovery compared to the Saline group, the 5 mL/kg Oxycyte showed a stronger improvement, with an average of 13-15 BBB score, while 2 mL/kg Oxycyte averaged 8-10 BBB score after day 42.

Histopathology: For molecular testing, Luxol blue reagent was used to determine the preservation of myelin and white matter, represented by the blue color, over a 6 week period in the three groups (Figure 2A). In the SCI group, the amount of myelin decreased over 6 weeks and spared 18-20% white matter on day 42. Saline (5 mL/kg) preserved more myelin and spared 28-30% white matter on day 42. However, Oxycyte (5mL/kg) preserved a lot more myelin and spared 50-52% white matter, serving as a stronger neuroprotectant over 6 weeks (Figure 2B). In addition, Tunel staining was used to indicate levels of apoptotic cell death. As dark brown spots denote apoptotic cell death, both Oxycyte groups had significantly less apoptotic cell deaths compared to the SCI and Saline groups after day 7 (Figure 3). Though, further studies need to be conducted to determine an optimal dose of Oxycyte to potentially reach better recovery.

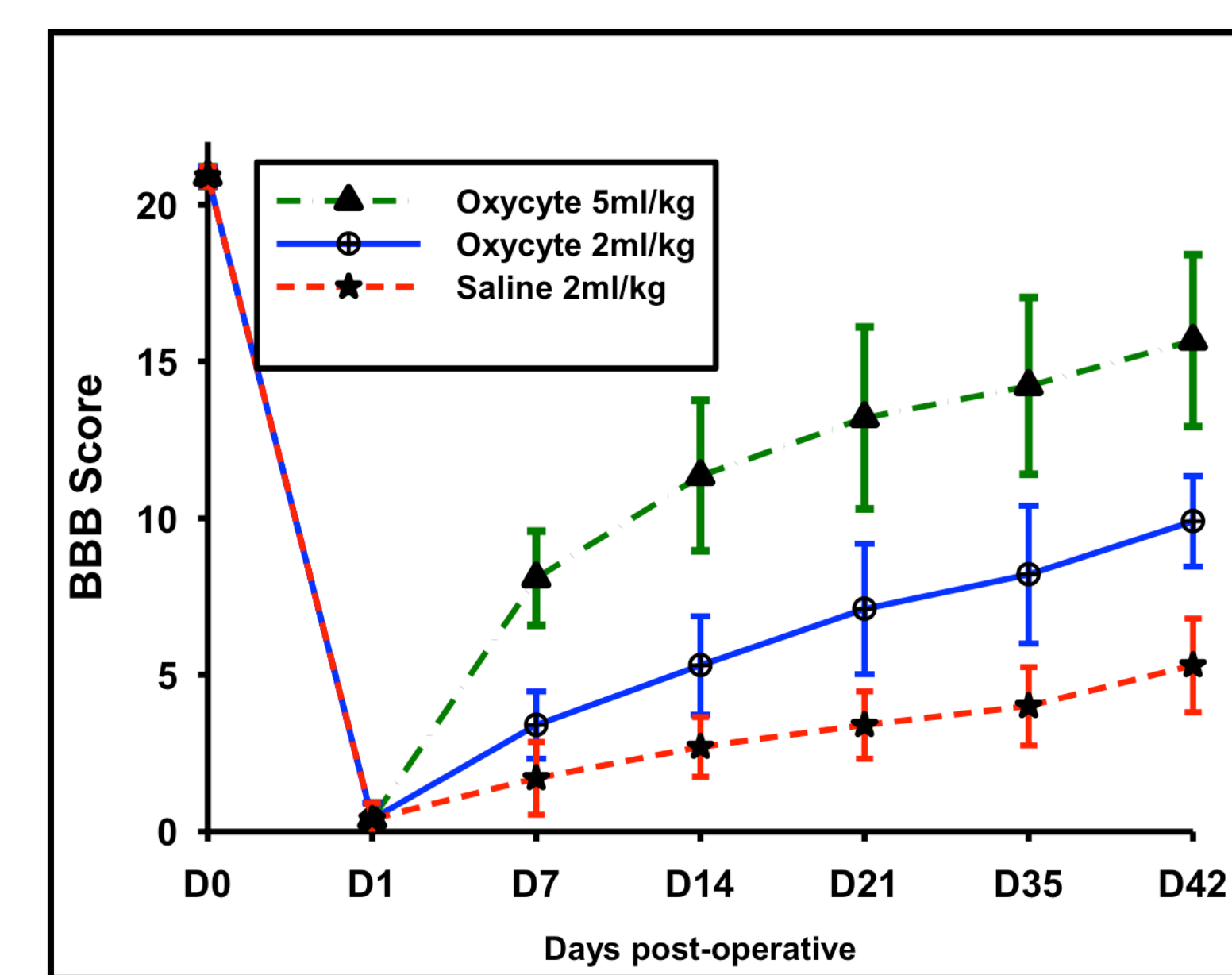


Figure 1: BBB score showing Oxycyte (5 mL/kg) significantly improved motor function compared to Saline

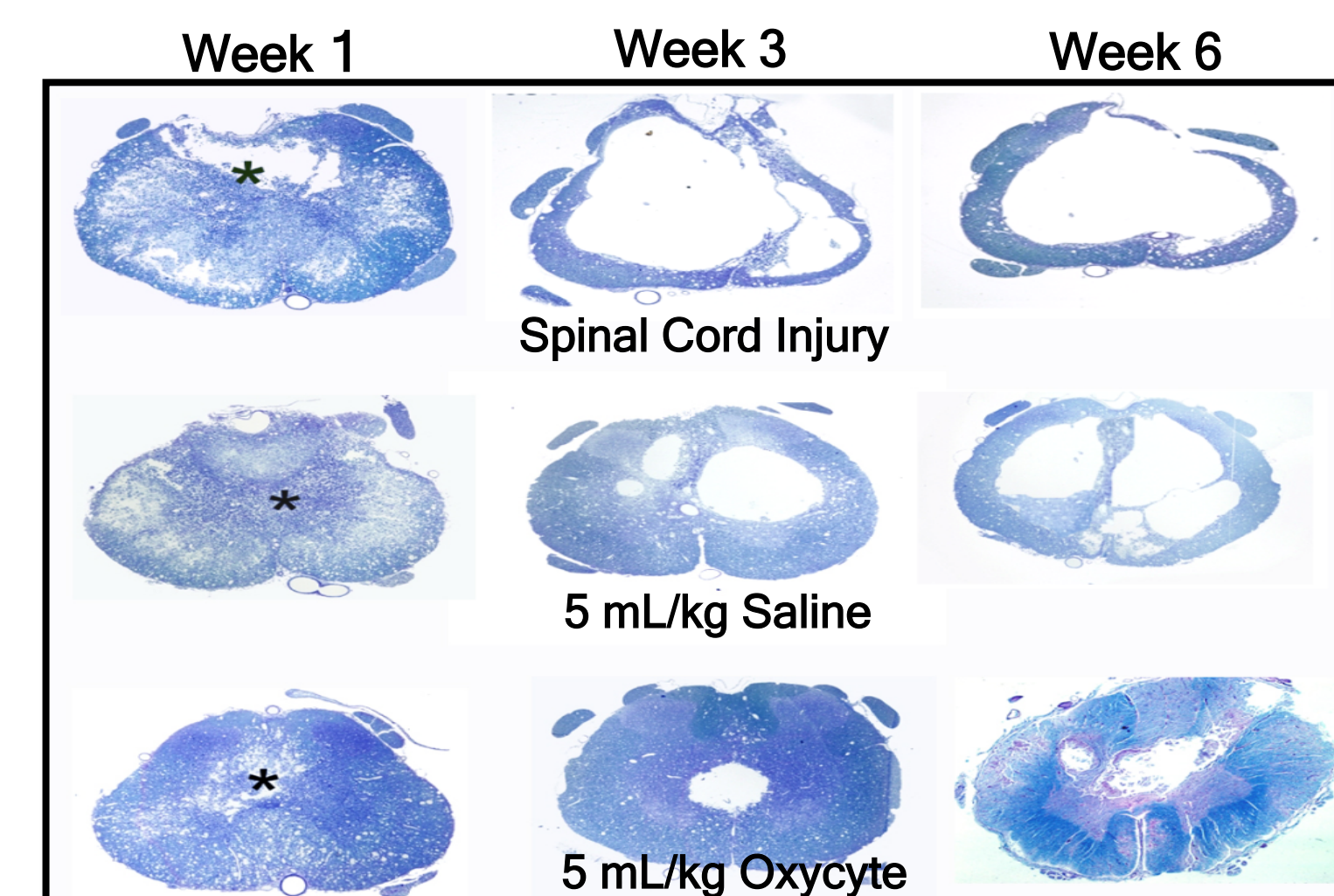


Figure 2A: Greater myelin preservation in 5mL/kg Oxycyte than SCI and Saline from Week 1 to Week 6

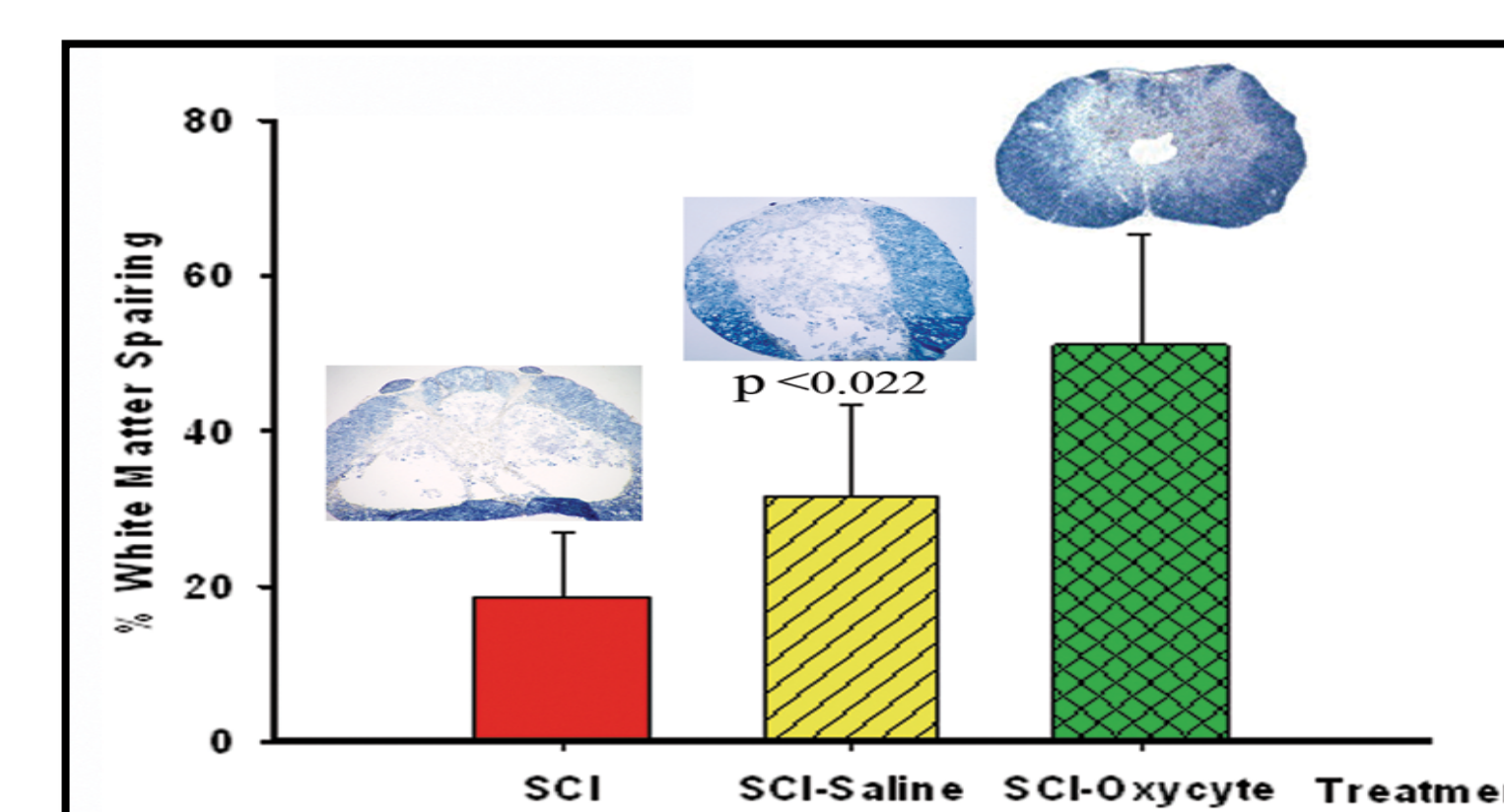


Figure 2B: 5mL/kg Oxycyte spared more white matter after 42 days

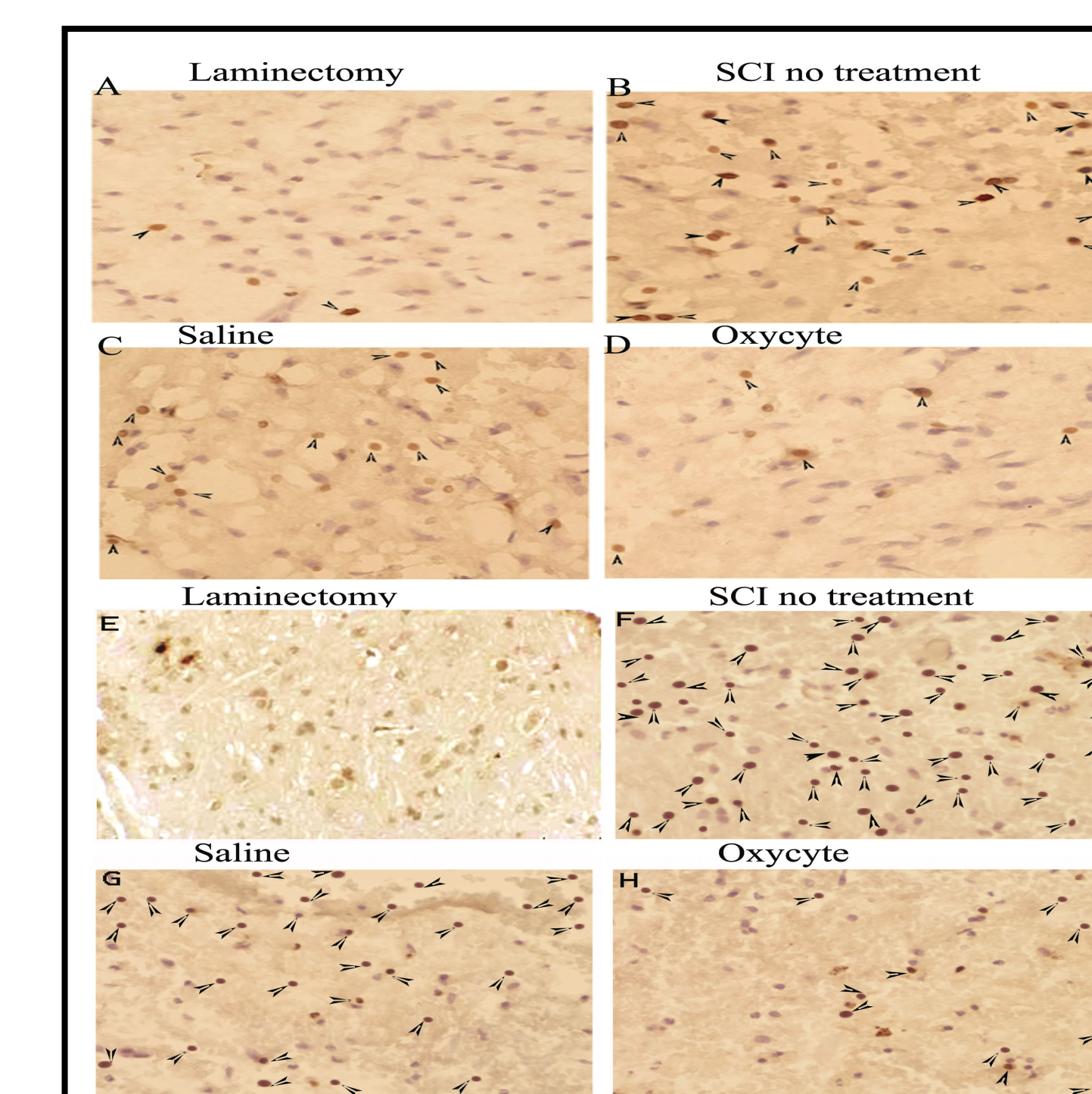


Figure 3: Less Amount of Apoptotic Cell Deaths in Oxycyte after 7 days

CONCLUSION

- Oxycyte improves motor function to a higher level of normality post SCI in comparison to Saline, according to the BBB scale and the incline plane test
- Oxycyte reduces lesion size, by preserving myelin sheath and white matter post SCI
- Oxycyte serves as a strong neuroprotectant and reduces more apoptotic cell deaths in comparison to Saline post SCI
- Optimal and efficacious dosage of Oxycyte needs to be further researched to apply in clinical setting

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