Electrophysiological assessment of retinal functions by ERG in Ischemia/Reperfusion (I/R) Allium cepa pre-treated mice

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ABSTRACT
Retinal disorders are the one of the most challenging and complex degenerative diseases that need to be addressed because of rapid increase in the number of affected individuals. Most of the available treatments strategies are inadequate to exert permanent solution to the patients. Therefore, as an alternative approach we wanted to test the efficacy of Allium cepa (A. cepa) in an Ischemia/Reperfusion (I/R) mouse model. We orally administered the aqueous extract of A. cepa at different dosages 100 mg/kg, 200 mg/kg, 300 mg/kg 24 hrs prior to the surgery. Electroretinogram (ERG) analysis was carried out at 7 day, 21 day, and 28 day after the surgery. ERG recording depicted that A. cepa administration is able to increase the implicit time but not at the statistically significant level for which larger sample size and deeper analysis is required.

doi: 10.38205/imcr.010118

KEY WORDS
Allium cepa
pretreatment
ERG
PPA
Retina

Introduction
Repeated failure in treating different neurodegenerative diseases have left no option for the researchers to think about the alternative approaches. Fruits and vegetables are the excellent alternative source to treat such disorders (1). Different studies suggest the potential role of fruits and vegetables in reducing the risk of degenerative diseases (2, 3). Flavonoids, a polyphenolic secondary metabolite are known to have antioxidant, anti-inflammatory, and anti-microbial properties. These flavonoids have been used in different in vitro and in vivo models and several studies suggests its protective role in degenerative diseases (4, 5).

Eye related disorders are most common degenerative disorders and have very limited scope of permanent cure. However, to overcome this, various traditional approaches like herbal remedy and homeopathy have been employed by ophthalmologist (6). Scientific studies have reported preventive role of onion on different eye related disorders like; Cata ract (7, 8), Age-related Macular Degeneration (AMD) (9).

Onion bulbs (Allium cepa L.) are the rich source of flavonoid, especially in quercetin. Different groups have elucidated the potential biological activity of A. cepa as neuroprotective, antioxidant, antiallergic, and anti-inflammatory (5, 10, 11). Published literature explains that onion extract (OE) lowers the total cholesterol content in rabbits (12) and in rats (13) fed with high cholesterol diet. Protective role of ethanolic extract of A. cepa was found in case of cognition impairments in streptozotocin induced rat diabetes model (14). The neuroprotective effect of flavonoid is known to exert by two processes: First, by interacting with lipid kinase thereby leading to the inhibition of the apoptosis. Second, by benefiting the vascular system (15).

Retinal ischemia which is a leading cause of blindness, results due to insufficient supply of blood to the retina and is known to be associated with several eye related disorders like glaucoma, diabetic retinopathy, and optic retinopathy (16). There have not been many studies on neuroprotective role of onion in rescuing the retinal ganglion cell death resulting due to retinal ischemia. Therefore, in the present study we have investigated the efficacy of aqueous extract of Allium cepa in rescuing the retinal functions depicted by electroretinogram (ERG) analysis. Ischemia/Reperfusion (I/R) induced retina injured mouse model was used to alter the retinal functions thereby affecting the vision.

ERG is a non-invasive technique used to depict the retinal functions. The ability of ERG to detect and isolate various signals from a different set of retinal neurons makes it a important tool for electrophysiological measures and this can be achieved by controlling/changing the stimulus, light intensity or adaptation, and parameters for data processing (17). Data acquisition in the form of wave pattern is a means to represent and distinguish different retinal cells activity. The wave pattern generally starts in the following pattern: “a-wave” is the first negative deflection and represents the primary retinal neurons (photoreceptors: rods and cones); “b-wave” is the...
positive peak which represents the bipolar cells (17); c-wave originates from retinal pigment epithelium (RPE) cells; oscillatory potentials (OP) originates from inner retina/amacrine cells (18). We used scotopic ERG (dark-adapted mice) for this purpose.

**Methods**

**Animals**

A C-57BL/6J male 8- to 10-week-old mouse was used for the experimentation purpose. The weight of the mice ranges from 25 g–30 g. Ethical approval was obtained from animal ethical committee (IAEC) of Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, India. The animals were kept in the sterile cages and temperature and humidity controlled facility of PGIMER animal house with no restriction to food and water. A 12 hr light/dark cycle was followed. The animals were divided into 4 groups (Figure 1).

**Experimental groups**

The complete experiment was carried according to the GLP guidelines at Neuroscience Research Laboratory (19, 20). The *A. cepa* extract preparation is detailed in our previous publication (21). Different dosages of *A. cepa* (100 mg/kg, 200 mg/kg, 300 mg/kg) was administered 24 hours prior to the surgery. Animals were divided into 4 different groups: group 1 (Injury alone), group 2 (Injury + 100 mg/kg *A. cepa* pretreatment), group 3 (Injury + 200 mg/kg *A. cepa* pretreatment), and group 4 (Injury + 300 mg/kg *A. cepa* pretreatment).

**Surgery**

Combination of Xylazine (50 mg/ml) / Ketamine (1:4 ratio) was used to anesthetize the mice. Intraperitoneal injection (IP) was administrated. Approximately 1.5 cm incision was made around the neck region. Initially, the CCA was exposed by retracting the muscles. The bifurcation was exposed and ECA was ligated with a fine suture. Further, the bifurcation of ICA was traced down and PPA was ligated with a 7.0 mm ethicon suture. The ligation was maintained for 2 hrs after that the ligated sutures were removed and mice was allowed to reperfuse (7 day, 21 day, and 28 day) under the sterile condition. The surgery was carried out under the Leica Stereozoom microscope.

**Electroretinography recordings**

Scotopic electroretinography (ERG) was performed to evaluate the function of retina caused due to 2 hrs of ischemia. The experiment was carried out using iWork ERG instrument (Dover, USA). The ERG data was recorded and evaluated using Labscribe software. ERG was done for all the 4 groups. This experiment was performed once the 24 hrs dark adaptation completed. Anesthesia was administrated intraperitoneally and the animals were kept on heating pad. Tropicamide and methylcellulose eye drops were used to dilate the pupils. Ground electrode was placed on tail, negative electrode in between the ears, and positive electrode was connected to the cornea (19). The readings were taken with flashes of light.
Results

Table 1 represents the implicit time and amplitude of both a- and b-waves depicted by ERG. Scotopic ERG was performed on 24 hr dark adapted mice. We recorded four important parameters, i.e., implicit time to a-wave, implicit time to b-wave, amplitude of a-wave, and amplitude of b-wave. At 7 day, the implicit time to a-wave was found to be 45 ± 5.78 for Injury alone, 35 ± 8.01 for 100 mg/kg pretreated, 44.85 ± 2.86 for 200 mg/kg pretreated, and 45.45 ± 1.72 for 300 mg/kg pretreated group. For injury alone group, the implicit time to a-wave was highest 43.55 ± 1.72 for 200 mg/kg, whereas for injury alone it was 42.25 ± 4.51 for 100 mg/kg group; 42.27 ± 1.62 for 200 mg/kg; and 36.95 ± 6.75 for 300 mg/kg group. For injury alone it was 42.25 ± 4.51, for 100 mg/kg it was 42.27 ± 1.62 and for 300 mg/kg it was 36.95 ± 6.75. With the increase in time points the we observed that at 28 day the implicit time to a wave also increased i.e. 51.13 ± 3.12, 43.33 ± 0.85, 44.33 ± 1.84, 46.2 ± 0.76 for injury alone, 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively. Positive wave i.e. the b-wave implicit time was found to be 88.27 ± 9.26 for injury alone at 7 day, whereas for 21 day it was 77.25 ± 7.67, and for 28 day it recorded 102.27 ± 5.72. For A. cepa pretreatment group's implicit time to b wave at 7 day was 88.8 ± 7.17 for 100 mg/kg, 90.8 ± 2.19 for 200 mg/kg and 92.3 ± 1.45 for 300 mg/kg.

Table 1: Electrotoretinogram (ERG) analysis for Injury alone, and A. cepa pretreated groups (100 mg/kg, 200 mg/kg, 300 mg/kg) at different time points (7 day, 21 day, 28 day) (A). a-wave implicit time; b-wave implicit time (B). a-wave amplitude; b-wave amplitude. The data is represented as mean ± SE. For Injury alone group sample size was (7 day (n = 3); 21 day (n = 4); 28 day (n = 3)); for 100 mg/kg pretreated group (7 day (n = 4); 21 day (n = 3); 28 day (n = 3)); for 200 mg/kg pretreated group (7 day (n = 4); 21 day (n = 4); 28 day (n = 3)); for 300 mg/kg pretreated group (7 day (n = 4); 21 day (n = 4); 28 day (n = 4)).

| Time/Groups | Implicit Time a (Time in ms ± SE) | Implicit Time b (Time in ms ± SE) |
|-------------|-----------------------------------|-----------------------------------|
| 7 day       | Injury 45 ± 5.78                  | Injury 88.27 ± 9.26               |
|             | 100 mg/kg 35 ± 8.01              | 100 mg/kg 88.8 ± 2.19             |
|             | 200 mg/kg 44.85 ± 2.86           | 200 mg/kg 90.8 ± 2.19             |
|             | 300 mg/kg 45.45 ± 1.72           | 300 mg/kg 92.3 ± 1.46             |
| 21 day      | Injury 42.25 ± 4.51               | Injury 77.25 ± 7.67               |
|             | 100 mg/kg 42.27 ± 1.62           | 100 mg/kg 88.8 ± 3.89             |
|             | 200 mg/kg 43.55 ± 1.72           | 200 mg/kg 88.3 ± 8.09             |
|             | 300 mg/kg 44.33 ± 1.84           | 300 mg/kg 89.3 ± 8.09             |
| 28 day      | Injury 51.13 ± 3.12               | Injury 102.27 ± 5.72              |
|             | 100 mg/kg 43.33 ± 0.85           | 100 mg/kg 79 ± 4.06               |
|             | 200 mg/kg 44.33 ± 1.84           | 200 mg/kg 82 ± 4.56               |
|             | 300 mg/kg 46.2 ± 0.76            | 300 mg/kg 89.6 ± 5.09             |

for 300 mg/kg. At 21 day we found slight decrease in the implicit time to b-wave i.e. 87.2 ± 8.25 (100 mg/kg), 86.4 ± 3.89 (200 mg/kg), 88.3 ± 8.09 (300 mg/kg). Similar trends where observed for 28 day where mean implicit time to b-wave was recorded as follows: 79 ± 4.06, 82 ± 4.56, and 89.6 ± 5.09 for 100 mg/kg, 200 mg/kg, 300 mg/kg respectively.

Further, the amplitude for both the waves was recorded. The amplitude of a-wave for injury alone was -0.198 ± 0.031 (7 day), -0.1025 ± 0.048 (21 day), -0.124 ± 0.017 (28 day); for 100 mg/kg pretreatment group it was -0.192 ± 0.045 (7 day), -0.363 ± 0.05 (21 day), -0.188 ± 0.026 (28 day); for 200 mg/kg pretreatment group it was -0.339 ± 0.071 (7 day), -0.205 ± 0.031 (21 day), -0.302 ± 0.029 (28 day); and for 300 mg/kg pretreatment group it was -0.595 ± 0.013 (7 day), -0.407 ± 0.024 (21 day), -0.356 ± 0.062 (28 day). Similarly, the mean amplitude of b-wave was as follows: 0.0163 ± 0.016, 0.0295 ± 0.024, 0.004 ± 0.038 as for injury alone group; 0.021 ± 0.03, 0.0673 ± 0.026, 0.0163 ± 0.006 for 100 mg/kg group; 0.007 ± 0.02, 0.0617 ± 0.012, 0.1076 ± 0.035 for 200 mg/kg group; 0.086 ± 0.007, 0.078 ± 0.020, 0.074 ± 0.044 for 300 mg/kg A. cepa pretreated group at 7, 21, and 28 day time points respectively. The details of significance level have been incorporated in the supplementary file (Supplementary Table 1).

Discussion

Retinal ischemia is a serious complication associated with glaucoma, diabetic retinopathy, and optic retinopathy (16). The associated condition may lead to blindness if appropriate and adequate treatments are not given on time. We have established a retinal ischemia mouse model by ligating two important arteries i.e. ophthalmic artery (PPA) and ECA (20). It's a 2 hr occlusion model followed by reperfusion for 3 different time points: 7, 21, and 28-day. Because of the repeated failure (24) and side effects (25) of the available commercial drugs, we wanted to test the alternative approach of testing
A. cepa (onion) as the pretreatment strategy to explore if it is able to improve the vision or not. Apart from its many biological benefits in the form of antioxidant, anti-inflammatory, and neuroprotective activity (as shown by previous studies) its common availability makes it an important biological product to be tested for its efficacy (5, 10, 11). For this, 3 different concentrations of aqueous extract of commonly used red onion was tested i.e. 100 mg/kg, 200 mg/kg and 300 mg/kg. The functional efficacy of A. cepa in the form of retinal wave analysis is very important in retinal degeneration cases and to the best of our knowledge it has not been previously reported in such models. According to standardized protocols the ERG recordings are depicted by three important factors: the instrument setup, intensity of the light stimulus, and animal state (26).

ERG recording depicted that implicit time to a-wave decreased in all the A. cepa administered groups except for slight increase in 300 mg/kg in comparison to the injury alone group for 7-day, however, this decrease was not statistically significant. Similarly, the implicit time to b-wave for both 21-day and 28-day increased throughout the A. cepa administered groups in comparison to the injury alone group. However, for 28-day, the implicit time to b-wave was highest for injury alone group followed by 300 mg/kg. From this, it can be said that A.capa administration is able to increase the implicit time but not at the statistically significant level for which larger sample size and deeper analysis is required. Delay in implicit time a-wave have previously been reported in diseased cases (27). Similarly, reduction in a-wave amplitude has also been reported in certain cases. Mixed results were obtained in case of both amplitude a- and b-wave. Amplitude a-wave was recorded to be decreased in most of the A. cepa administered groups with respect to the injury alone group. Further, b-wave amplitude was highest at 7 day for 100 mg/kg; at 21 day for 300 mg/kg, and at 28 day for 200 mg/kg. So, from this data it can be concluded that A. cepa may able to improve the retinal functions depicted by ERG analysis.

Limitations of the study

Though we have tried to minimise the limitation, factors such as intensity of light stimulus, temperature/humidity, anesthesia, ocular environment, adaptive state, and other technical difficulties affecting the ERG recordings cannot be ruled out. Large sample size and more group like: Injury+ PBS/solvent comparison is needed.

Acknowledgments

Authors would like to acknowledge the Ministry of AYUSH, Govt. of India for providing the funding (Z.28015/106/2014-HPC (EMR)-AYUSH-A).

Authors Contribution

SK: Experimentation, data analysis, original writing
AA: Conceptualization, editing of the manuscript, securing funding
RS: Co-conceptualization
SK: Co-conceptualization
VS: Experimentation

Ethical statement

All experiments were performed after getting the approval from Institutional Animal Ethical Committee (IAEC) via approval no: 67/IAEC/390R.

Conflicts of interests

The authors declare that they have no conflict of interest.

Source of funding

Thanks to ministry of AYUSH file No. (Z.28015/106/2014-EMR-AYUSH-A) Government of India

Received Date: 10-02-20; Revised Date: 12-02-20
Accepted Date: 26-02-20

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## Supplementary Table 1

### POST HOC TESTS

#### 1. IMPLICIT TIME A-WAVE

**Multiple Comparisons**

| 7 Day | (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|-------|-----------|-----------|-----------------------|------------|------|-------------------------|-------------|-------------|
| Scheffe | Injury | Hundred | 10.0000 | 7.6475 | .646 | | -15.089 | 35.089 |
| | Two Hundred | .1500 | 7.6475 | 1.000 | | | -24.939 | 25.239 |
| | Three Hundred | -4500 | 7.6475 | 1.000 | | | -25.539 | 24.639 |
| | Hundred | Injury | -10.0000 | 7.6475 | .646 | | -35.089 | 15.089 |
| | Two Hundred | -9.8500 | 7.0803 | .602 | | | -33.077 | 13.377 |
| | Three Hundred | -10.4500 | 7.0803 | .557 | | | -33.677 | 12.777 |
| | Two Hundred | Injury | -1.5000 | 7.6475 | 1.000 | | -25.239 | 24.939 |
| | Hundred | .98500 | 7.0803 | .602 | | | -13.377 | 33.077 |
| | Three Hundred | -6000 | 7.0803 | 1.000 | | | -23.827 | 22.627 |
| | Three Hundred | Injury | .45000 | 7.6475 | 1.000 | | -24.639 | 25.539 |
| | Hundred | 10.4500 | 7.0803 | .557 | | | -12.777 | 33.677 |
| | Two Hundred | .60000 | 7.0803 | 1.000 | | | -22.627 | 23.827 |
| | Three Hundred | Injury | -10.4500 | 7.0803 | .364 | | -29.735 | 8.835 |
| | Hundred | .98500 | 7.0803 | .602 | | | -23.200 | 20.633 |
| | Three Hundred | -6000 | 7.0803 | 1.000 | | | -19.885 | 18.685 |

### 1.2. Dependent Variable: Implicit Time A-Wave: 21 Day

| 21 Day | (I) Group | (J) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|-------|-----------|-----------|-----------------------|------------|------|-------------------------|-------------|-------------|
| Scheffe | Injury | Hundred | -.01667 | 6.68074 | 1.000 | | -21.9335 | 21.9001 |
| | Two Hundred | -1.30000 | 6.18516 | .997 | | | -18.9910 | 18.9910 |
| | Three Hundred | 5.30000 | 6.18516 | .863 | | | -14.9910 | 25.5910 |
| | Hundred | Injury | .91667 | 6.68074 | 1.000 | | -21.9001 | 21.9335 |
| | Two Hundred | -1.28333 | 6.68074 | .998 | | | -23.2001 | 20.6335 |
| | Three Hundred | 5.31667 | 6.68074 | .887 | | | -16.6001 | 27.2335 |
| | Two Hundred | Injury | 1.30000 | 6.18516 | .997 | | -18.9910 | 21.9510 |
| | Hundred | 1.28333 | 6.68074 | .998 | | | -20.6335 | 23.2001 |
| | Three Hundred | 6.60000 | 6.18516 | .770 | | | -13.6910 | 26.8910 |
| | Three Hundred | Injury | -5.30000 | 6.18516 | .863 | | -25.5910 | 14.9910 |
| | Hundred | -5.31667 | 6.68074 | .887 | | | -27.2335 | 16.6001 |
| | Two Hundred | -6.60000 | 6.18516 | .770 | | | -26.8910 | 13.6910 |
| | Three Hundred | Injury | 5.30000 | 6.18516 | .736 | | -11.5473 | 22.1473 |
| | Hundred | 5.31667 | 6.68074 | .774 | | | -12.8805 | 23.5138 |
| | Two Hundred | 6.60000 | 6.18516 | .601 | | | -10.2473 | 23.4473 |
### 1.3. Dependent Variable: Implicit Time A-Wave: 28 Day

| 28 Day | (I) Group3 | (J) Group3 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|--------|------------|------------|-----------------------|------------|------|------------------------|
|        |            |            | Lower Bound           | Upper Bound|      |                        |
| Scheffe| Injury     | Hundred    | 7.80000               | 2.58314    | .085 | ~0.9932                | 16.5932            |
|        | Two Hundred|            | 6.80000               | 2.58314    | .145 | ~1.9932                | 15.5932            |
|        | Three Hundred|        | 4.93333               | 2.41630    | .308 | ~3.2919                | 13.1586            |
|        | Hundred    |            | ~7.80000              | 2.58314    | .085 | ~16.5932               | 9.932              |
|        | Two Hundred|            | ~1.00000              | 2.58314    | .984 | ~9.7932                | 7.7932             |
|        | Three Hundred|        | ~2.86667              | 2.41630    | .711 | ~11.0919               | 5.3586             |
|        | Two Hundred|            | ~6.80000              | 2.58314    | .145 | ~15.5932               | 1.9932             |
|        | Hundred    |            | 1.00000               | 2.58314    | .984 | ~7.7932                | 9.7932             |
|        | Three Hundred|        | ~1.86667              | 2.41630    | .895 | ~10.0919               | 6.3586             |
|        | Three Hundred|        | ~4.93333              | 2.41630    | .171 | ~1.9099                | 11.7766            |
| Dunnett t (2-sided)*| Injury | Three Hundred| 4.93333               | 2.41630    | .500 | ~9.0999                | 3.9766             |
|        | Three Hundred|        | ~2.86667              | 2.41630    | .540 | ~9.0999                | 3.9766             |
|        | Two Hundred |            | ~1.86667              | 2.41630    | .796 | ~8.7099                | 4.9766             |

### 2. IMPLICIT TIME B-WAVE

#### 2.1. Dependent Variable: Implicit Time B-Wave: 7 Day

| 7 Day | (I) Group1 | (J) Group1 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|-------|------------|------------|-----------------------|------------|------|------------------------|
|        |            |            | Lower Bound           | Upper Bound|      |                        |
| Scheffe| Injury     | Hundred    | ~5.3333               | 8.02095    | 1.000 | ~26.8468               | 25.7802            |
|        | Two Hundred|            | ~2.5333               | 8.02095    | .991  | ~28.8468               | 23.7802            |
|        | Three Hundred|        | ~4.03333              | 8.02095    | .967  | ~30.3468               | 22.8802            |
|        | Hundred    |            | ~5.3333               | 8.02095    | 1.000 | ~25.7802               | 26.8468            |
|        | Two Hundred|            | ~2.00000              | 7.42596    | .995  | ~26.3616               | 22.3616            |
|        | Three Hundred|        | ~3.50000              | 7.42596    | .973  | ~27.8616               | 20.8616            |
|        | Two Hundred|            | ~2.53333              | 8.02095    | .991  | ~23.7802               | 28.8468            |
|        | Hundred    |            | ~2.00000              | 7.42596    | .995  | ~22.3616               | 26.3616            |
|        | Three Hundred|        | ~1.50000              | 7.42596    | .998  | ~25.8616               | 22.8616            |
|        | Three Hundred|        | ~4.03333              | 8.02095    | .967  | ~22.2802               | 30.3468            |
|        | Hundred    |            | 3.50000               | 7.42596    | .973  | ~20.8616               | 27.8616            |
|        | Two Hundred |            | 1.50000               | 7.42596    | .998  | ~22.8616               | 25.8616            |
| Dunnett t (2-sided)*| Injury | Three Hundred| ~4.03333              | 8.02095    | .924  | ~25.8810               | 17.8143            |
|        | Three Hundred|        | ~3.50000              | 7.42596    | .936  | ~23.7270               | 16.7270            |
|        | Two Hundred |            | ~1.50000              | 7.42596    | .994  | ~21.7270               | 18.7270            |
### 2.2. Dependent Variable: Implicit Time B-Wave: 21 Day

| 21 Day | (I) Group2 | (J) Group 2 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|--------|------------|-------------|------------------------|------------|------|------------------------|
|        |            |             | Upper Bound | Lower Bound |
| Scheffe| Injury     | Hundred     | -9.95000    | 10.50654    | .826 | -44.4177               |
|        |            | Two Hundred | -9.15000    | 10.50654    | .828 | -41.0609               |
|        |            | Three Hundred | -11.05000 | 9.72716    | .736 | -42.9609               |
|        | Hundred    | Injury      | 9.95000     | 10.50654    | 1.000 | -33.6677               |
|        |            | Two Hundred | 8.00000     | 10.50654    | 1.000 | -35.5677               |
|        |            | Three Hundred | -1.10000  | 10.50654    | 1.000 | 33.5677               |
|        | Two Hundred| Injury      | 9.15000     | 9.72716     | .828 | -22.5177               |
|        |            | Hundred     | -8.00000    | 10.50654    | 1.000 | -35.2677               |
|        |            | Three Hundred | -1.90000 | 9.72716    | .998 | -33.8109               |
|        | Three Hundred| Injury   | 11.05000    | 9.72716     | .736 | -20.8609               |
|        |            | Hundred     | 1.10000     | 10.50654    | 1.000 | -33.3677               |
|        |            | Two Hundred | 1.90000     | 9.72716     | .998 | -30.0109               |
| Dunnett t (2-sided)a | Injury | Three Hundred | -11.05000 | 9.72716 | .557 | -37.5451               |
|        |            | Hundred     | -1.10000    | 10.50654    | .999 | -29.7180               |
|        |            | Two Hundred | -1.90000    | 9.72716     | .995 | -28.3951               |

### 2.3. Dependent Variable: Implicit Time B-Wave: 28 Day

| 28 Day | (I) Group3 | (J) Group 3 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|--------|------------|-------------|------------------------|------------|------|------------------------|
|        |            |             | Upper Bound | Lower Bound |
| Scheffe| Injury     | Hundred     | 232.6667    | 7.36009     | .070 | -1.7875                |
|        |            | Two Hundred | 202.6667    | 7.36009     | .123 | -4.7875                |
|        |            | Three Hundred | 126.6667 | 6.88473    | .388 | -10.7694               |
|        | Hundred    | Injury      | -23.2667    | 7.36009     | .070 | -48.3209               |
|        |            | Two Hundred | -3.00000    | 7.36009     | .982 | -28.0542               |
|        |            | Three Hundred | -10.60000 | 6.88473    | .529 | -34.0361               |
|        | Two Hundred| Injury      | -20.2667    | 7.36009     | .123 | -45.3209               |
|        |            | Hundred     | -3.00000    | 7.36009     | .982 | -22.0542               |
|        |            | Three Hundred | -7.60000 | 6.88473    | .752 | -31.0361               |
|        | Three Hundred| Injury    | -12.6667    | 6.88473     | .388 | -36.1027               |
|        |            | Hundred     | 10.60000    | 6.88473     | .529 | -12.8361               |
|        |            | Two Hundred | 7.60000     | 6.88473     | .752 | -15.8361               |
| Dunnett t (2-sided)a | Injury | Three Hundred | 12.6667    | 6.88473    | .230 | -6.8318                |
|        |            | Hundred     | -10.60000   | 6.88473     | .349 | -30.0984               |
|        |            | Two Hundred | -7.60000    | 6.88473     | .591 | -27.0984               |
3. AMPLITUDE a-WAVE

3.1. Dependent Variable: Amplitude a-Wave: 7 Day

| 7 Day     | (I) Group 1 | (J) Group 1 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|-----------|-------------|-------------|-----------------------|------------|------|------------------------|
|           |             |             |                       |            |      |                        |
| Injury    | Hundred     | -.0062500   | .0701467              | 1.00       |      | -.236373               |
|           | Two Hundred | .1410000    | .0701467              | .310       |      | -.089123               |
|           | Three Hundred | .3965000*  | .0701467              | .001       |      | .166377                |
|           |             |             |                       |            |      | .266623                |
| Scheffe   | Injury      | .0062500    | .0701467              | 1.00       |      | -.233873               |
|           | Two Hundred | .4725000    | .0649432              | .222       |      | -.065802               |
|           | Three Hundred | .4027500*  | .0649432              | .001       |      | .189698                |
|           |             |             |                       |            |      | .615802                |
| Two Hundred | Injury     | -.1410000   | .0701467              | .310       |      | -.371123               |
|           | Two Hundred | .1472500    | .0649432              | .222       |      | -.360302               |
|           | Three Hundred | .3965000*  | .0649432              | .001       |      | .189698                |
|           |             |             |                       |            |      | .615802                |
| Three Hundred | Injury     | -.4027500*  | .0649432              | .018       |      | -.468552               |
|           | Two Hundred | .3965000*   | .0649432              | .000       |      | .205433                |
|           |             |             |                       |            |      | .587657                |
| Dunnett t | Injury      | .0062500    | .0701467              | 1.00       |      | -.236373               |
|           | Two Hundred | .1032500    | .0528513              | .332       |      | -.070134               |
|           | Three Hundred | .3055000*  | .0528513              | .001       |      | .132116                |
|           |             |             |                       |            |      | .478884                |
| (2-sided)^a| Injury     | -.3965000*  | .0701467              | .310       |      | -.626623               |
|           | Two Hundred | .1472500    | .0649432              | .222       |      | -.360302               |
|           | Three Hundred | .3965000*  | .0649432              | .001       |      | .189698                |
|           |             |             |                       |            |      | .615802                |
| Three Hundred | Injury     | -.4027500*  | .0649432              | .018       |      | -.468552               |
|           | Two Hundred | .3965000*   | .0649432              | .000       |      | .205433                |
|           |             |             |                       |            |      | .587657                |
|           |             |             |                       |            |      | .587657                |

*. The mean difference is significant at the 0.05 level.

3.2. Dependent Variable: Amplitude a-Wave: 21 Day

| 21 Day     | (I) Group 2 | (J) Group 2 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|------------|-------------|-------------|-----------------------|------------|------|------------------------|
|            |             |             |                       |            |      |                        |
| Injury     | Hundred     | .2615000*   | .0570859              | .007       |      | .074224                |
|            | Two Hundred | .1032500    | .0528513              | .332       |      | .276634                |
|            | Three Hundred | .3055000*  | .0528513              | .001       |      | .132116                |
|            |             |             |                       |            |      | .478884                |
| Scheffe    | Injury      | -.2615000*  | .0570859              | .007       |      | -.448776               |
|            | Two Hundred | .1032500    | .0528513              | .332       |      | -.070134               |
|            | Three Hundred | .3055000*  | .0528513              | .001       |      | .132116                |
|            |             |             |                       |            |      | .478884                |
| Hundred    | Injury      | -.1582500   | .0570859              | .108       |      | -.345526               |
|            | Two Hundred | .0440000    | .0570859              | .896       |      | .143276                |
|            | Three Hundred | .1582500   | .0570859              | .332       |      | .276634                |
|            |             |             |                       |            |      | .70134                 |
| Two Hundred | Injury     | -.1032500   | .0528513              | .332       |      | -.276634               |
|            | Two Hundred | .1582500    | .0570859              | .108       |      | -.029026               |
|            | Three Hundred | .2022500*  | .0528513              | .021       |      | .028866                |
|            |             |             |                       |            |      | .375634                |
| Three Hundred | Injury     | -.3055000*  | .0528513              | .001       |      | -.478884               |
|            | Two Hundred | .0440000    | .0570859              | .896       |      | -.231276               |
|            |             |             |                       |            |      | .143276                |
| Dunnett t  | Injury      | .3055000*   | .0528513              | .000       |      | .161542                |
| (2-sided)^a| Two Hundred | .0440000    | .0570859              | .789       |      | -.111492               |
|            | Three Hundred | .2022500*  | .0528513              | .007       |      | .058292                |
|            |             |             |                       |            |      | .346208                |

*. The mean difference is significant at the 0.05 level.
### 3.3. Dependent Variable: Amplitude a-Wave: 28 Day

| 28 Day | (I) Group 3 | (J) Group 3 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|--------|-------------|-------------|------------------------|------------|-----|-------------------------|-------------|-------------|
| Injury | Hundred     | .0640000    | .0637237               | .800       |     |                        | −.152920    | .280920     |
|        | Two Hundred | .1776667    | .0637237               | .117       |     |                        | −.039253    | .394586     |
|        | Three Hundred | .2315000*  | .0596081               | .026       |     |                         | .028590     | .434410     |
| Scheffe| Hundred     | −.0640000   | .0637237               | .800       |     |                        | −.280920    | .152920     |
|        | Two Hundred | .1136667    | .0637237               | .413       |     |                        | −.103253    | .330586     |
|        | Three Hundred | .1675000   | .0596081               | .114       |     |                         | −.035410    | .370410     |
|        | Injury       | −.1776667   | .0637237               | .117       |     |                        | −.394586    | .039253     |
|        | Two Hundred | −.1136667   | .0637237               | .413       |     |                        | −.330586    | .103253     |
|        | Three Hundred | .0538333   | .0596081               | .844       |     | −.149076               | .256743     | .434410     |
|        | Hundred      | .2315000*   | .0596081               | .026       |     | −.001318               | .336318     | .725651     |
| Dunnett t (2-sided) | Injury | Three Hundred | .2315000* | .0596081 | .010 | .062682 | .400318 |
|        | Hundred Three Hundred | .1675000 | .0596081 | .052 | −.001318 | .336318 |
|        | Two Hundred Three Hundred | .0538333 | .0596081 | .717 | −.114985 | .222651 |

* The mean difference is significant at the 0.05 level.

### 4. AMPLITUDE b-WAVE

### 4.1. Dependent Variable: Amplitude b-Wave: 7 Day

| 7 Day | (I) Group 1 | (J) Group 1 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | Lower Bound | Upper Bound |
|-------|-------------|-------------|------------------------|------------|-----|-------------------------|-------------|-------------|
| Injury | Hundred     | −.0046667   | .0343660               | .999       |     | −.117407               | .108074     | .100778     |
|        | Two Hundred | .0093333    | .0343660               | .994       |     | −.103407               | .122074     | .120047     |
|        | Three Hundred | −.0696667  | .0343660               | .303       |     | −.182407               | .043074     | .150578     |
| scheffe| Hundred     | .0046667    | .0343660               | .999       |     | −.108074               | .117407     | .100778     |
|        | Two Hundred | .0140000    | .0318167               | .978       |     | −.090378               | .118378     | .039378     |
|        | Three Hundred | −.0650000  | .0318167               | .297       |     | −.169378               | .039378     | .103407     |
|        | Injury       | −.0093333   | .0343660               | .994       |     | −.122074               | .103407     | .100778     |
|        | Two Hundred | −.0140000   | .0318167               | .978       |     | −.118378               | .090378     | .039378     |
|        | Three Hundred | −.0790000  | .0318167               | .165       |     | −.183378               | .025378     | .181378     |
| Dunnett t (2-sided) | Injury | Three Hundred | .0093333 | .0343660 | .160 | .063274 | .23940 |
|        | Hundred Three Hundred | −.0650000 | .0318167 | .156 | −.151663 | .021663 |
|        | Two Hundred Three Hundred | −.0790000 | .0318167 | .075 | −.165663 | .007663 |
### 4.3. Dependent Variable: Amplitude b-Wave: 21 Day

| 21 Day | (I) Group 2 | (J) Group 2 | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|--------|-------------|-------------|-----------------------|------------|------|------------------------|
|        |             |             |                       |            |      | Lower Bound | Upper Bound |
| Scheffe| Injury      | Hundred     | -.0378333             | .0306341   | .685 | -1.38331   | .062665     |
|        |             | Two Hundred | -.0322500             | .0283616   | .735 | -.125293   | .060793     |
|        |             | Three Hundred | -.0405000          | .0283616   | .439 | -.141543   | .044543     |
|        | Hundred     | Injury      | .0378333              | .0306341   | .685 | -.062665   | .138331     |
|        |             | Two Hundred | .0055833              | .0306341   | .998 | -.094915   | 1.06081     |
|        |             | Three Hundred | -.0106667         | .0306341   | .989 | -.111165   | .089831     |
|        | Two Hundred | Injury      | .0322500              | .0283616   | .735 | -.060793   | .125293     |
|        |             | Hundred     | -.0055833             | .0306341   | .998 | -.106081   | .094915     |
|        |             | Three Hundred | -.0162500          | .0283616   | .953 | -.109293   | .076793     |
|        | Three Hundred | Injury  | .0485000              | .0283616   | .439 | -.044543   | .141543     |
|        |             | Hundred     | .0106667              | .0306341   | .972 | -.094109   | .072775     |
|        |             | Two Hundred | .0162500              | .0283616   | .972 | -.093502   | .061002     |
| Dunnett t (2-sided)* | Injury | Three Hundred | -.0485000          | .0283616   | .261 | -.125752   | .028752     |
|        |             | Hundred     | -.0106667             | .0306341   | .972 | -.094109   | .072775     |
|        |             | Two Hundred | -.0162500             | .0283616   | .972 | -.093502   | .061002     |

* Dunnett t-tests treat one group as a control, and compare all other groups against it.