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BIOCHEMICAL AND SPECTROSCOPIC ANALYSIS OF MICE BRAIN AFTER BLAST TRAUMATIC BRAIN INJURY

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ABSTRACT

Objective: This study investigates the biochemical and spectroscopic alterations in mice brain after blast traumatic brain injury (bTBI), focusing on molecular alterations detected by Fourier Transform Infrared (FTIR) spectroscopy.

Methods: Male and female Balb/c mice were subjected to blast traumatic brain injury (bTBI) using an improvised explosive device (IED). Neurological impairment, neurobehavioral deficits, metabolic changes, brain water content, infarct size, and antioxidant enzyme activity were assessed to characterize the TBI- induced alterations. FTIR spectroscopy was employed to analyze the molecular composition of the brain and liver tissues.

Results: The Neurological Severity Scores (NSS) revealed varying degrees of injury, with mild, moderate, or severe impairment. Neurobehavioral assessments using open field, beam walk, and elevated plus maze tests demonstrated altered exploratory behavior and anxiety-related behavior deficits in TBI mice. The metabolic assessment revealed changes in body weight, blood count, and blood glucose levels. Brain water content measurement and infarct size estimation indicated the presence of cerebral edema and tissue damage after bTBI. Assessment of antioxidant enzyme activity revealed altered levels of catalase, glutathione, superoxide dismutase, and nitrate in the brain, suggesting oxidative stress and inflammation. Furthermore, FTIR spectroscopy analysis provided insights into the molecular alterations that occur in the brain and liver homogenate after bTBI.

Conclusion: This study highlights the critical role of oxidative stress in the pathophysiology of bTBI and demonstrates that FTIR spectroscopy is a promising method for detecting biochemical alterations in brain and liver. These findings may contribute to the development of more sensitive diagnostic approaches and therapeutic strategies for blast-induced brain injury.

1. INTRODUCTION

Blast traumatic brain injury (bTBI) is a significant public health concern that can lead to a range of physical, cognitive, and emotional symptoms (Guley, 2017). The worldwide prevalence of bTBI is a topic of ongoing research and surveillance, particularly among military personnel who are at an increased risk of exposure to explosive blasts. According to the United States Department of Defense, there were over 383,000 cases of TBI among US military personnel between 2000 and 2020, with many of these cases being blast related. However, data on the prevalence of blast TBI in other countries and in civilian populations are limited. Pakistan has been affected by various forms of conflict and terrorism (Nizami et al., 2018). Consequently, the risk of exposure to explosive blasts is high, particularly among military personnel and civilians living in conflict affected areas. According to a report by the Pakistani government, over 22,000 terrorism-related incidents occurred in the country between 2001 and 2013, resulting in over 50,000 deaths and many more injuries. Although the exact number of bTBI cases resulting from these incidents is unknown, it is likely that this type of injury has affected many individuals, and Pakistan is home to many refugees and internally displaced persons. These populations may be at an increased risk for blast Research, suggesting that the prevalence of bTBI may increase globally, particularly in regions affected by conflict and terrorism (Kovacs et al., 2014).

Based on clinical examination using the GCS bTBI is classified as mild, moderate or severe. In mild TBI, loss of consciousness is 30 min or less, and in moderate TBI, the patient is lethargic. Severe TBI results in an extended period of unconsciousness, long-term complications, or death (Dixon, 2017). Reviewing clinical and neuropathological findings from blast-exposed persons helps to explain how blast waves cause traumatic injury. bTBI shows significant acute differences from mild TBI. (Ling et al., 2009) emphasized the major differences between blast and blunt TBI. First, bTBI causes acute cerebral edema, subarachnoid hemorrhage pseudoaneurysms, and delayed arterial vasospasm.

Animal models, particularly mice, have been used extensively to study blast TBI and its effects on behavior (Liu et al., 2015; O'Connor et al., 2011; ÖCAL, 2019) . These models allow for controlled exposure to blast waves and detailed analysis of the resulting cognitive and behavioral deficits. Many studies have used a controlled experimental design in which mice were exposed to a single or repeated blast injury and then tested for behavioral deficits compared with a control group of mice (Wang et al., 2011). Blast injuries are typically induced using a specialized device that delivers a controlled blast wave to an animal's head. The severity of blast injuries can vary depending on a number of factors, including the intensity and duration of the blast wave, distance from the source of the blast, and individual differences in susceptibility and resilience. Severe blast TBI can cause profound and long-lasting symptoms, such as coma, seizures, paralysis, and even death (Dixon, 2017).

One of the most significant consequences of TBI is the disruption of biochemical processes that occur within the brain. Enzymes play a crucial role in these processes, and their activity can be affected by TBI (Cheng et al., 2019; Rakib, Al-Saad, Ahmed, et al., 2021). Recent research study investigated the activity of brain enzymes after bTBI. The study found that the activity of enzymes involved in glucose metabolism was significantly decreased, whereas the activity of enzymes involved in oxidative stress was increased. This suggests that bTBI leads to an imbalance in the metabolic processes within the brain (Abdul-Muneer et al., 2015). It has also been reported that bTBI alters brain metabolism. A research study found that TBI patients had lower cerebral glucose metabolism than the controls (Xu et al., 2021). CT and MRI were used to diagnose brain damage. CT and MRI detect bleeding or hemosiderin. CT and MRI require large

amounts of red blood cells for bleeding. Unless a major blood vessel ruptures. These technologies failed to detect multiple, extensive, and tiny axonal damages, which could cause serious cognitive abnormalities. Unlike histological staining, FTIR is a "state-of-the-art" bio diagnostic and bioimaging tool for quick medical and biological studies. The molecule absorbs electromagnetic radiation because of atom vibrations at a given wavenumber and forms an infrared (IR) band. The vibrational wavenumber of the IR spectrum provides biomolecules with a biological and biophysical fingerprint. FTIR spectroscopy is easy, sensitive, accurate, reproducible, nondestructive, and fast. FTIR imaging spectroscopy has been used to study pathological alterations in breast cancer, stroke, PD, and other neurodegenerative illnesses (Summers et al., 2017). FTIR spectroscopy is a simple and fast technique. No dyes or reagents are needed to identify brain structures, and the procedure is nondestructive; thus, the sample can be studied using other methods (Rakib, Al-Saad, Ahmed, et al., 2021).One study by Rakib et al., 2021 used FTIR spectroscopy to investigate changes in the molecular composition of brain tissue after blast TBI in a rat model. Researchers found significant changes in the amide I and II bands, which are associated with protein content, as well as lipid bands. They concluded that FTIR spectroscopy might be a useful tool for monitoring biochemical changes in the brain after blast TBI. Many other research studies used FTIR spectroscopy to investigate changes in the molecular composition of brain tissue after nervous disorders in a mouse model. The researchers found significant changes in the lipid bands as well as in the amide I and II bands. Thus, FTIR can be a quick and reproducible method for characterizing the biochemical makeup of distinct brain regions and provides a useful platform for molecular-level brain modifications in neurological diseases (Ali et al., 2018; Du et al., 2013; Rakib, Al-Saad, Ustaoglu, et al., 2021; Summers et al., 2017; Surowka et al., 2017).

1.2. Aim and Objectives

The aim of this study was to elucidate biochemical and biomolecular alterations in brain regions of mice after bTBI. Following are the objectives of the study:

1. To understand the pathophysiology of bTBI in mice model.
2. To analyze various parameters after and before bTBI in mice model.
3. Biochemical analysis of mice brain after bTBI.
4. Spectroscopic analysis of mice brain and liver after bTBI.

2. Materials and Methods

2.1. Ethical Approval

All procedures followed NIH guidelines for the care and use of experimental animals and were approved by the Ethical and Animal Care and Usage Committee, SA-CIRBS, Faculty of Sciences, IIUI.

2.2. Animals and Experimental Design

Male and female Balb/c mice (25–30 g) were obtained from NIH Islamabad, housed four per cage at 25 ± 2°C under a 12 h light/dark cycle, with food and water ad libitum. Animals were acclimatized for one week before blast traumatic brain injury (bTBI) induction.

2.3. TBI Induction Model

An open-field blast exposure model was used. Mice without anesthesia were placed 3 m from an improvised explosive device. A Mercury TSL01 decibel meter recorded sound pressure levels. After exposure, animals were returned to the housing facility.

2.4. Neurological Assessment

Neurological Severity Score (NSS) was recorded at 0, 3, 7, and 14 days post-injury. Scores

ranged from 0 (normal) to 10 (maximal deficit), evaluating motor, sensory, reflex, and balance functions.

2.5. Neurobehavioral Tests

Motor and anxiety-related behaviors were evaluated using the beam walk, open field, and elevated plus maze tests. Beam walk assessed crossing time and slips on a 2 cm-wide, 60 cm-long beam. The open-field test measured ambulation, central area time, and rearing. The elevated plus maze recorded open/closed arm entries and time spent in each.

2.6. Metabolic Assessments

Body weight was recorded at baseline and on days 3, 7, and 14 post-bTBI. Blood was collected via cardiac puncture for hematological analysis. Blood glucose was measured using a glucometer.

2.7. Brain Edema

Brains were weighed (wet weight) at day 3 post-injury, dried at 80°C for 72 h, and reweighed. Brain water content was calculated as: brain water content = ([wet weight - dry weight]/wet weight) 100%.

2.8. Infarct Size Measurement

Brains were sliced into six 2 mm sections and stained in 0.05% TTC at 37°C for 20 min. After PBS washing and 4% formalin fixation, slices were imaged and infarct size quantified using SAT software.

2.9. Antioxidant Enzyme Activity

Catalase activity was measured at 240 nm using H_2O_2 degradation. Glutathione (GSH) activity was determined at 412 nm with DTNB. Lipid peroxidation (LPO) was quantified via TBARS assay at 535 nm. Superoxide dismutase (SOD) activity was determined by pyrogallol auto-oxidation inhibition at 312 nm. Nitrite levels were measured using the Griess reaction at 540 nm.

2.10. Reactive Oxygen Species (ROS)

ROS production was determined by DCFH-DA oxidation to DCF in brain homogenates, measured fluorometrically, and expressed as moles of DCF/min/mg protein.

2.11. FTIR Spectroscopy

Brain and liver homogenate samples were homogenized in PBS and analyzed using FTIR spectroscopy to determine protein and lipid composition.

2.12. Statistical Analysis

Data were analyzed using GraphPad Prism 8.0. One-way or two-way ANOVA with Tukey's multiple comparison test was used to determine statistical significance ($p < 0.05$).

3. Results

3.1. Sound Pressure Level of Improvised Explosive device (IEd)

The graph shows the change in sound pressure level at varying distances from the explosion point, as measured in an open field using the IEd issued by SA-CIRBS, IIUI. The IEd generated a high sound pressure level, and the measuring instrument had limitations. Consequently, the most precise measurement achievable was at 20 m from the explosion point. At this distance, the sound pressure level recorded for the IEd was 125dB. The uncertainty of the microphone reading in this range was ±1 dB.

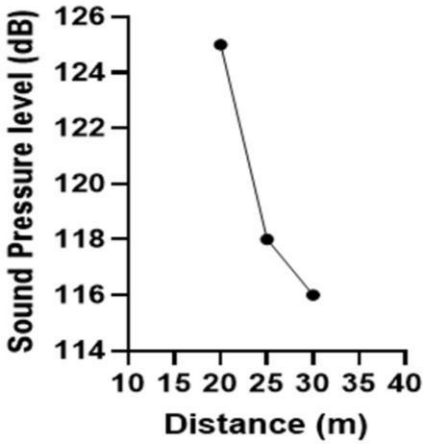


Figure 3.1. Sound pressure level (dB) of IED issued by the SA-CIRBS, IIUI in an open area.

3.2. Neurological Severity Score (NSS)

The graph shows the NSS of mice after the blast exposure. The control group gained 0 NSS points and successfully performed all 10 tasks. Data were analyzed using a Two-way ANOVA. Tukey's multiple comparison tests showed a highly significant difference among control males (0.000±0.000) and bTBI males (4.278 ± 0.3889), while bTBI males compared to bTBI females showed no significance. The graphical data showed significant changes in the NSS after the blast explosion [$F(3, 57) = 244.2$, $P < 0.0001$].

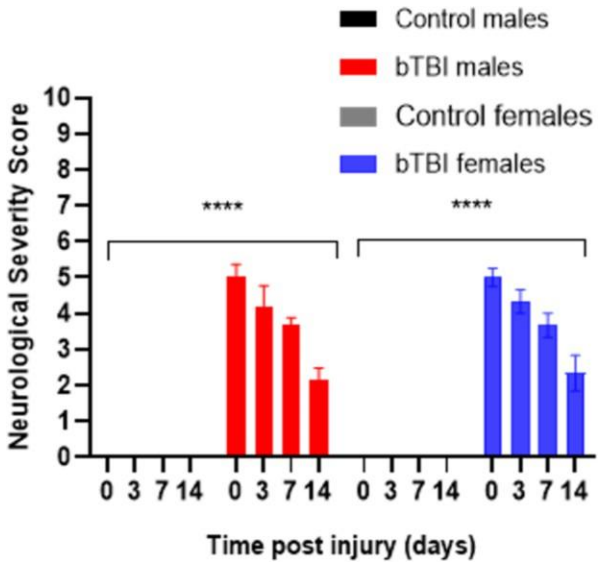


Figure 3.2. Effect of bTBI on NSS during experimentation period. Values are expressed as Mean ± SEM (n=6). ****P < 0.0001.

3.3. Neurobehavioral Assessment

3.3.1. Balance Beam Test

The graphs show the effect of bTBI on motor coordination and balance in the mice. Figure 4.3.

(A) represent the average time (sec) of each group taken to cross the beam. The data were analyzed by Two-way ANOVA, and Tukey's multiple comparison test showed a significant difference among control males (7.667±0.3849) and bTBI males (23.44 ± 2.328), whereas bTBI males compared to bTBI females showed no significance. The graphical data showed significant change in the beam walk after the blast explosion [F (3, 24) = 127.4, P < 0.0001]. Figure 4.3. (B) shows the number of foot slips during balance beam walking after blast exposure. The data were analyzed by Two-way ANOVA, and Tukey's multiple comparison test showed a significant difference among control males (0.7778±0.2222) and bTBI males (4.000± 0.3333), whereas bTBI males compared to bTBI females showed no significance. The graphical data showed significant change in the beam walk after the blast explosion [F (3, 24) = 41.70, P < 0.0001].

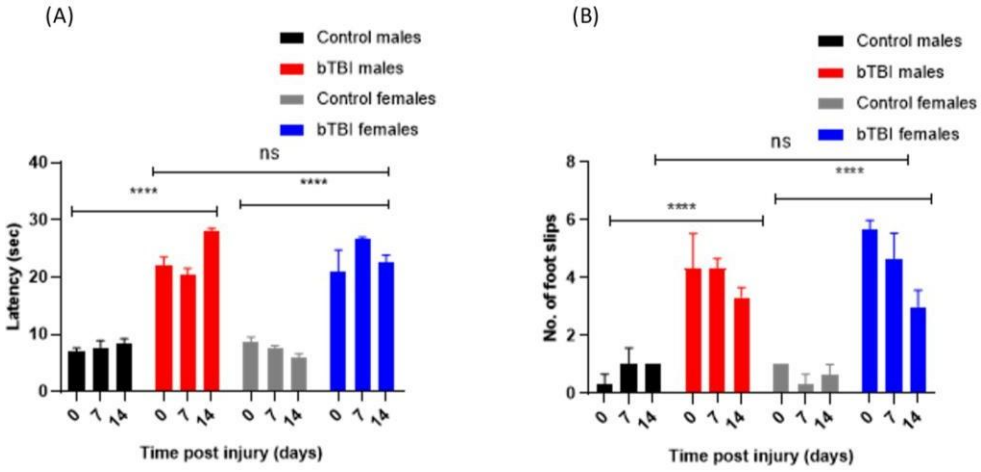


Figure 3.3. Effects of bTBI on balance and coordination. (A) shows the average time (sec) of each group taken to cross the beam. (B) shows the number of foot slips during balance beam walking after blast exposure. Results are represented in Mean ± SEM (n= 3). ****$P < 0.0001$ and ns: not significant.

3.3.2. Open Field Test (OFT)

The graphs show the effect of bTBI on locomotory, and exploratory behavior assessed by OFT. The data were analyzed by Two way of ANOVA, Tukey's multiple comparisons test. Figure 4.4 (A) shows that mean differences in crossing lines [F (3, 84) = 52.89, P <0.0001]. The number of line crossing was higher in control males (103.3± 1.918) as compared to bTBI males (50.88± 2.895). Similarly, the number of line crossing was

higher in control females (103.9± 1.593) as compared to bTBI females (75.79± 1.158). Figure 4.4. (B) shows rearing behavior of mice [F (3, 75) = 50.00, P<0.0001]. The rearing behavior of control (16.00±0.4732) was higher as compared to bTBI males (2.525± 1.338). Similarly, the rearing behavior of control (15.96± 0.4640) was higher as compared to bTBI females (4.892±1.857). Figure 4.4 (C) represents the time spent in center (sec) [F (3, 16) = 7.976, P=0.0018]. The control animals entered more times in the central zone as contrast to other groups.

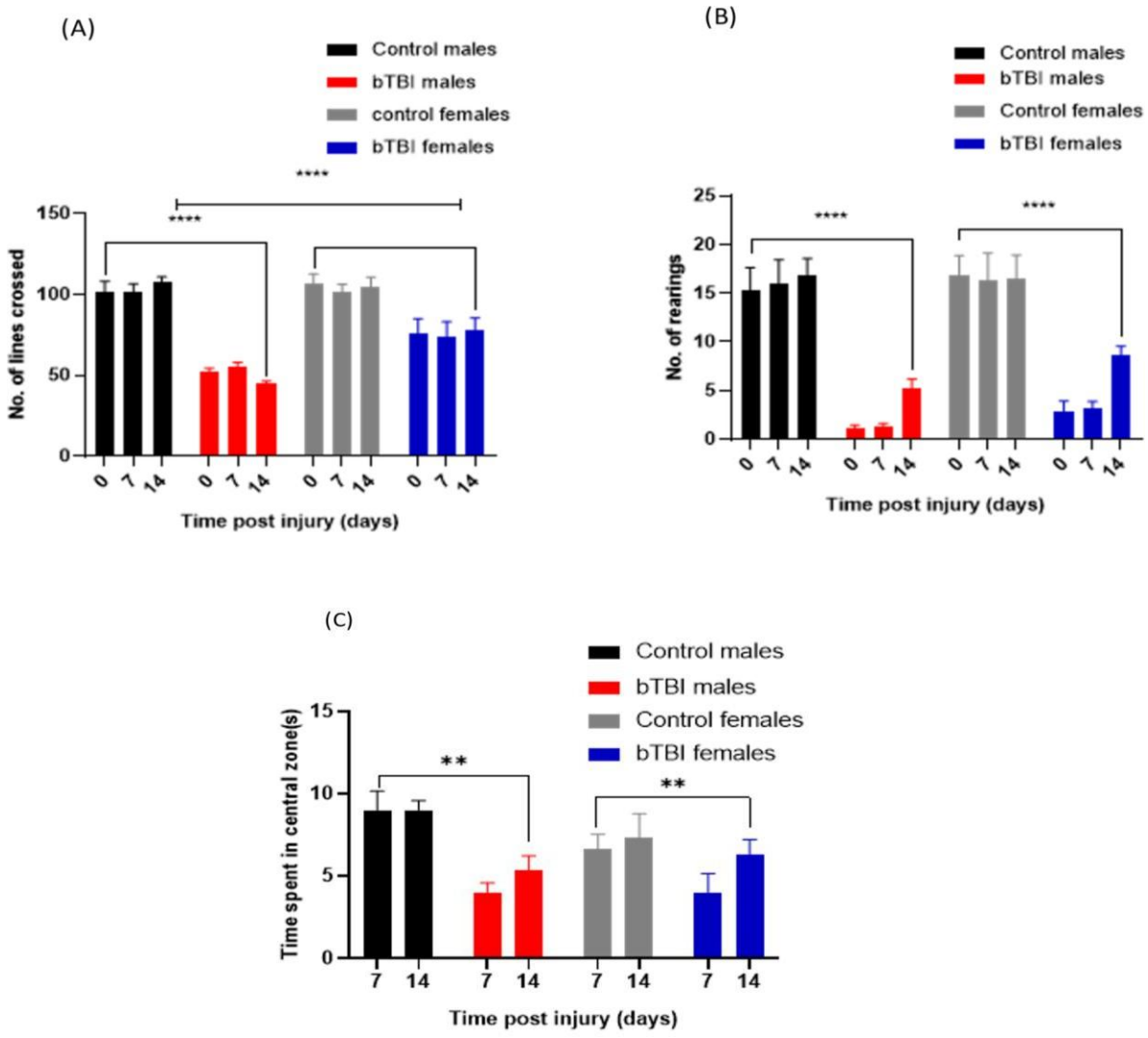


Figure 3.4. Effect of bTBI on locomotory and exploratory behavior assessed by Open Field test. (A) shows the number of line crossing of animals in Open Field. (B) shows the number of rearing in Open Field, (C) shows time spent in the center by animals in Open Field. Results are represented in Mean ± SEM (n=3). $^{**}P < 0.01$, and $^{****}P < 0.0001$.

3.3.3. Elevated plus Maze Test

The graphs shows the effect of bTBI on anxiety-like behaviour accessed by elevated plus maze test. The data were analyzed by Two-way ANOVA, Tukey's multiple comparisons test. Figure 4.5 (A) represent the number of entries in closed arm that control group have less entries (28.00± 1.667) as compared to bTBI males (34.67± 3.000). Similarly, the number of entries were less in control females (30.33± 2.000) as compared to bTBI females (36.17± 2.500). The results showed significance difference among the groups [F (3, 16) = 17.76, P<0.0001]. Figure 4.5. (B) represents the time spent in closed arm that control group spent less time (106.2± 26.50) as compared to bTBI males (153.8± 3.500). Similarly, the time spent in closed arm was lower in control females (64.3± 12.00) as compared to bTBI females (150.0± 2.082). The results showed significance difference among the groups [F (3, 16) = 22.18, P<0.0001]. Figure 4.5. (C) represents the number of entries in open arm that control group had higher number of entries (11.83± 1.500) as compared to bTBI males (5.333± 1.333). Similarly, the number of entries in open arm was higher in control females (8.833± 0.5000) as compared to bTBI females (3.333± 0.6667). The results showed significance difference among the groups [F (3, 16) = 30.91, P <0.0001]. Figure 4.5. (D) represents the time spent in open arm that control group spent more time (107.2±6.833) as compared to bTBI males (26.17± 3.500). Similarly, the time spent in open arm was higher in control females (99.00± 4.667) as compared to bTBI females (29.17± 2.833). The results showed significance difference among the groups [F (3, 16) = 47.03, P<0.0001].

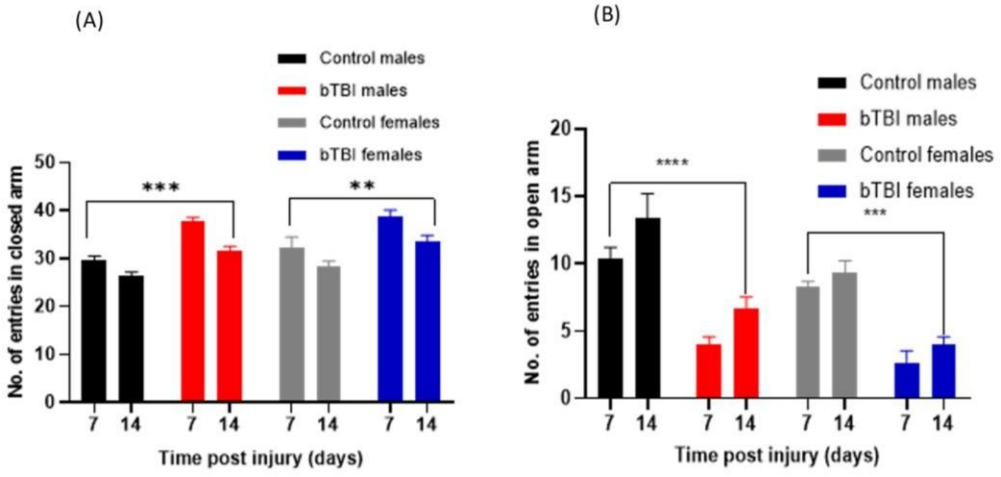


Figure 3.5. Effect of bTBI on anxiety-like behavior accessed by Elevated plus maze test. (A) represents the number of entries in closed arm. (B) represents the time spent in closed arms. Results are represented in Mean ± SEM (n= 3). ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001.

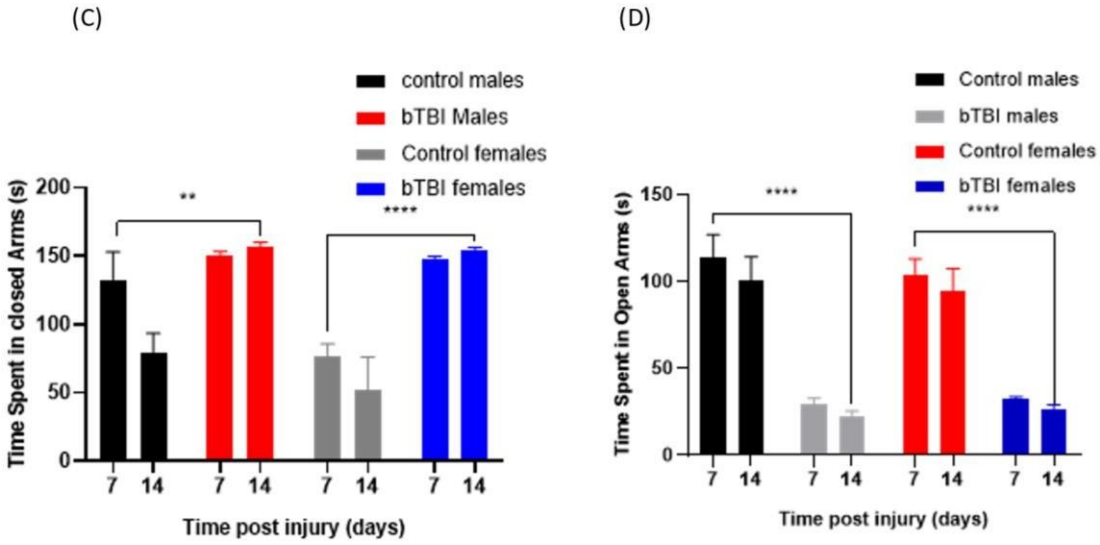


Figure 3.6. Effect of bTBI on anxiety-like behavior accessed by Elevated plus maze test. (C) represents the number of entries in open arm (D) represents the time spent in open arms. Results are represented in Mean ± SEM (n= 3). $^{**}P < 0.01$ and $^{****}P < 0.0001$.

3.4. Metabolic Tests Assessment

3.4.1. Body Weight

Results showed the change in body weight of mice after blast exposure. Data were analyzed using a Two-way ANOVA. Bonferroni's multiple comparison tests showed a highly significant difference among control males (36.83±0.6386) and bTBI males (40.78±3.081). Similarly, Bonferroni's multiple comparison test showed a highly significant difference among control females (37.68± 0.7434) and bTBI females (41.93±3.022). The graph showed significant change in body weight after blast exposure [F (3, 24) = 21.48, P < 0.0001].

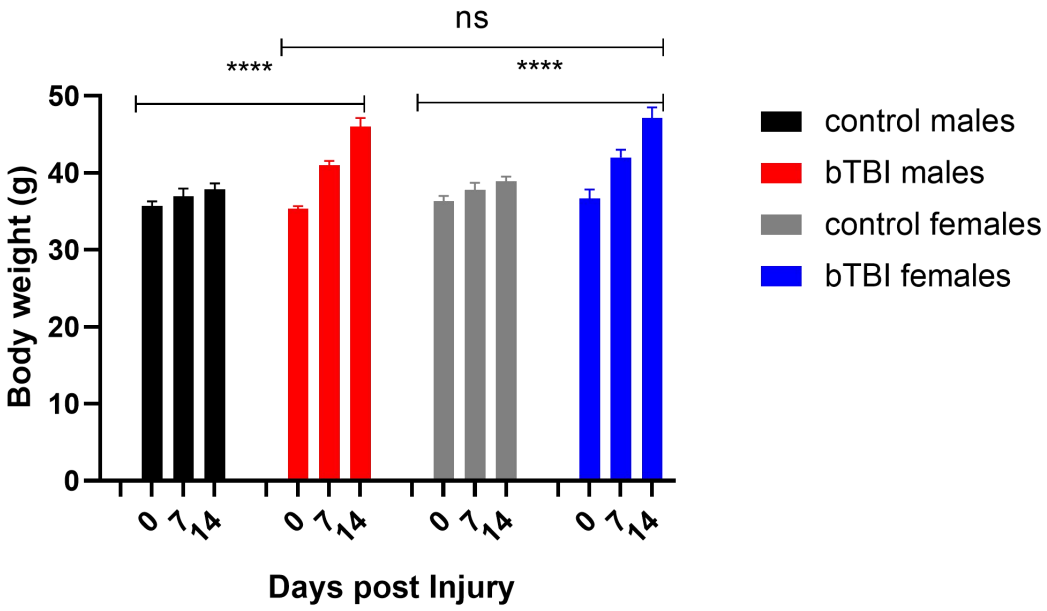
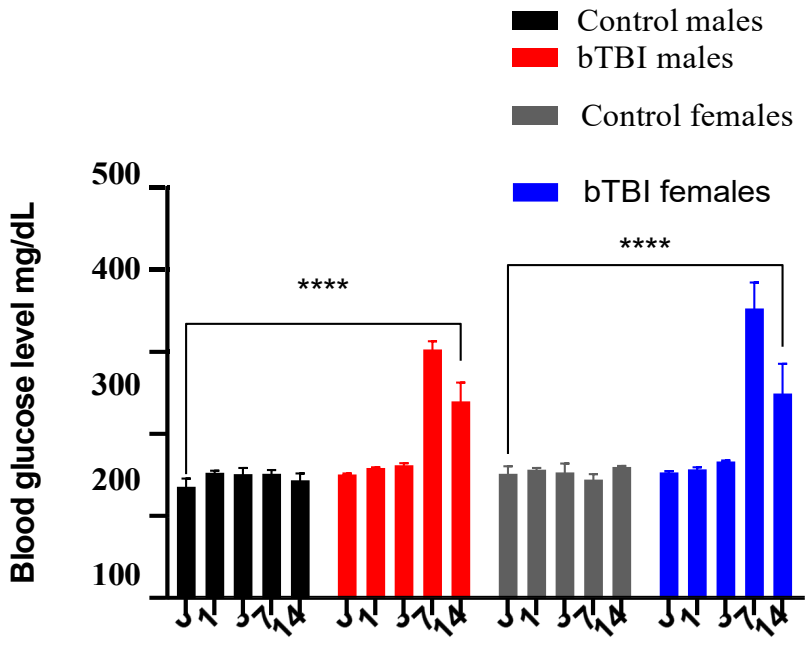


Figure 3.7. Effect of bTBI on body weight among the groups before and after the blast explosion.

Results represented in Mean ± SEM (n=3). ****$P < 0.0001$.

3.4.2. Blood Glucose levels

The blood glucose levels (mg/dL) of mice were measured using a glucometer at 0d, 1d, 7d, and 14d after bTBI. Two-way ANOVA analysis revealed significant differences among the groups ($F(3, 40) = 45.40$, $p < 0.0001$). Tukey's multiple comparison tests indicated that blood glucose levels were significantly higher in bTBI males (189.9±28.62) compared to control males (146.9±3.212), and in bTBI females (202.9±37.99) compared to control females (153.1±2.596). However, bTBI males compared to bTBI females showed no significance.



Time post injury (days)

Figure 3.8. Blood Glucose level (mg/dL) among groups during the experimentation period after bTBI. Values are expressed as mean + SEM(n=3). ****$P < 0.0001$.

3.5. Hematological Parameters

The complete blood counts of male and female mice after bTBI were evaluated. bTBI males had significantly higher levels of WBCs and RBCs compared to the control male, but significantly decreased Hb levels and platelet counts. Whereas, bTBI female mice had significantly decreased WBCs counts, RBCs, Hb levels, and high platelet counts compared to the control group.

Table 2 Blood parameter results after bTBI in mice

Parameter	Control male	bTBI male	Control female	bTBI female
White blood cells (WBCs)	4 x103/uL	6.9 $x10^3$/uL	3.5 $x10^3$/uL	2.8 $x10^3$/uL
Red blood cells (RBCs)	5 $x10^6$/uL	6.9 $x10^6$/uL	7 $x10^6$/uL	6.7 $x10^6$/uL
Hemoglobin (Hb)	15 g/dL	11.6 g/dL	13 g/dL	10.7 g/dL
Platelets (x103/µL)	284× 10^3 /µL	752 x103/uL	248× 10^3 /µL	641 $x10^3$/uL

3.6. Effect of bTBI on Brain Water Content (BWC):

Results of BWC showed a significant injury effect on the measure of brain water content at 7d post-injury [F (3, 8) = 131.4, P < 0.0001]. Tukey's multiple comparison test showed a significant difference between control males (80.09± 0.03283) and bTBI males (88.36± 0.3584). Similarly, there was also a significant difference between control females (80.30± 0.3512) and bTBI females (87.03± 0.5717). However, there was no statistically significant difference found between the bTBI male and bTBI female groups.

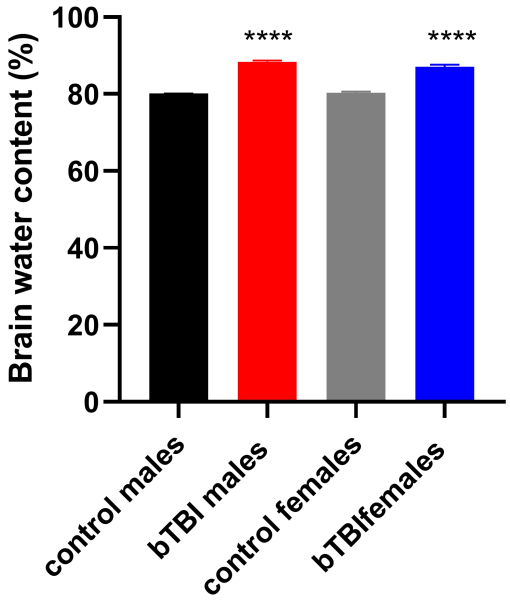


Figure 3.9. Effect of bTBI on brain water content as an indicator of brain edema. Results are expressed as mean + SEM (n=3). ****$P < 0.0001$.

3.7. The Effect of bTBI on ROS levels

The results showed a significant increase in ROS levels in the TBI group compared with the control group, F (3, 8) = 17.82, P < 0.0007]. Tukey's multiple comparison test showed a significant difference between control males (3.358± 0.008718) and bTBI males (5.684±0.8649). While bTBI females compared to control females show no significant difference.

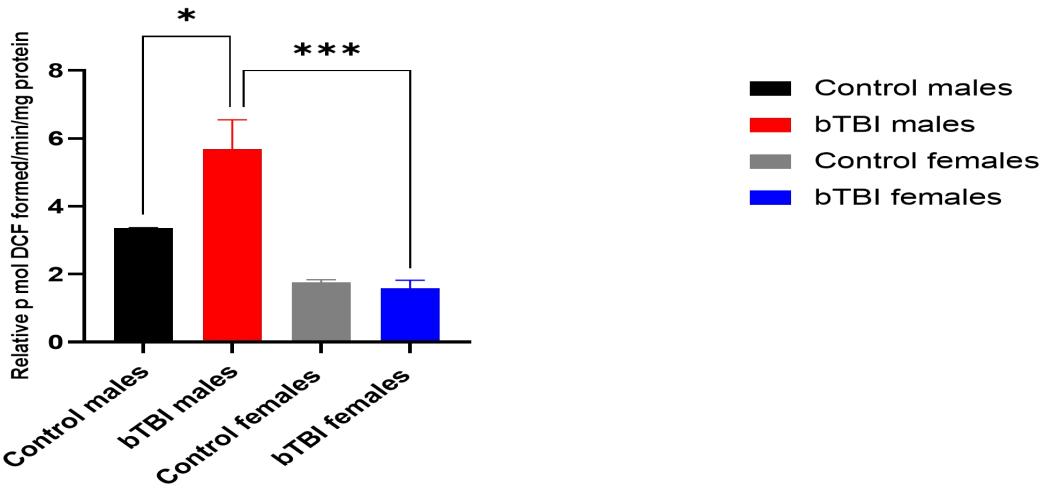


Figure 3.10. ROS levels in the brain of mice at 7 days of bTBI. Values are expressed as mean± SEM (n=3). *$P < 0.05$, and **$P < 0.01$.

3.8. TTC Staining

Results of TTC staining showed a significant increase in the infarct size (%) in the TBI group compared to control group [F (3, 20) = 33.32, P < 0.0001]. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. The result showed a highly significant difference between control males (0.000±0.000) and bTBI males (0.5150± 0.06587). Similarly, the infarct size (%) of bTBI females was found to be (0.6067 ±0.09175), which was significantly higher than that of the control group (0.000±0.000). bTBI males showed no significant difference from bTBI females.

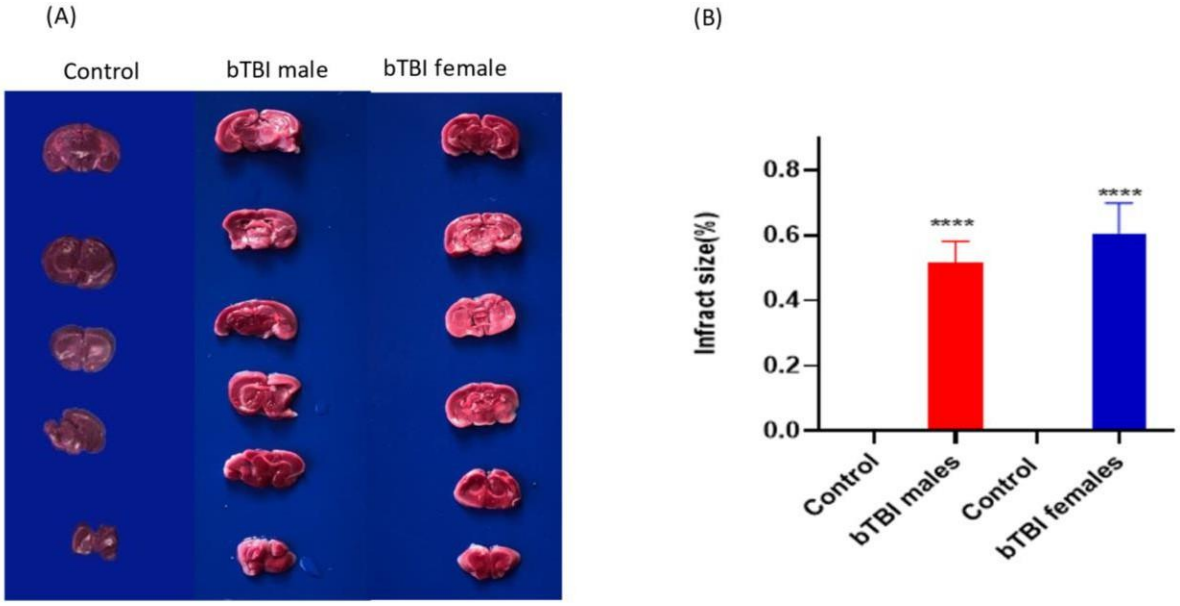


Figure 3.11. The cerebral infract size (%) was detected by the TTC staining method after bTBI. (A) TTC staining was used in mouse brain tissue sections at 3d after TBI. The infarct area appears white. (B) Analysis of volume of infraction of a mouse brain. Values are expressed as mean ± SEM(n=3 ****$P < 0.0001$.

3.9. Effect of bTBI on the enzymatic activities in the Mice Brain:

The effect of bTBI on the enzymatic activities of CAT, GSH, NO, SOD and LPO in the brain of mice after 7d of blast explosion was estimated. Data were analyzed using one-way (ANOVA) followed by Tukey's multiple comparison test. The results showed a significant decrease in CAT and SOD activity in the TBI group compared to the control group. Catalase activity was significantly decreased in the TBI groups (control males:60.72 ± 1.794, bTBI males:3.280 ± 0.1769; control females:64.35 ± 4.306, bTBI

females:2.090 ± 0.2800; F (3, 8) = 218.8, P < 0.0001). Similarly, SOD activity was significantly decreased in the TBI groups (control males:100.3 ± 6.007, bTBI males:50.67 ± 8.655; control females:103.3 ± 3.844, bTBI females:51.75 ± 7.751; F(3, 8) = 18.42, P < 0.0006). Furthermore, NO levels were elevated in male mice with TBI (control males:15.05 ± 0.3457, bTBI males:65.93 ± 13.92; F(3, 8) = 11.90, P = 0.0026), while GSH levels were reduced in both male and female mice with TBI (control males:98.33 ± 8.413, bTBI males:61.67 ± 7.265; control females:98.33 ± 5.207, bTBI females:40.33 ± 1.453; F(3, 8) = 21.54, P = 0.0003). However, there was no significant change in lipid peroxidation (LPO) activity between the TBI and control groups [F (3, 8) = 1.720, P = 0.2398)].

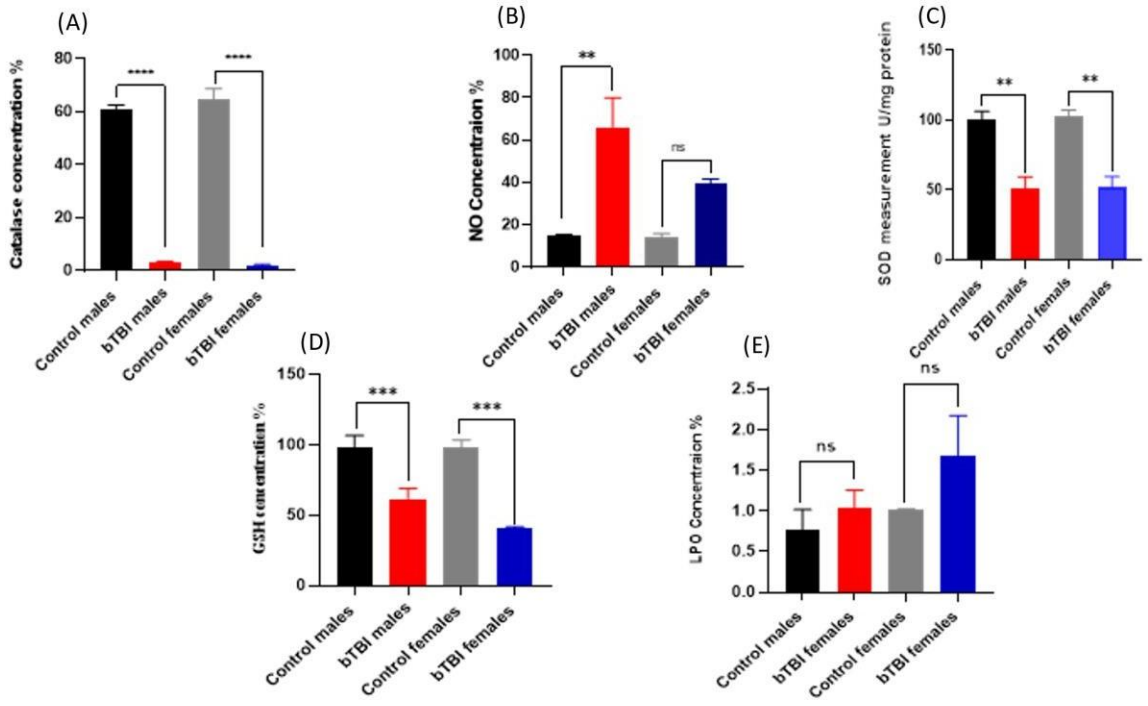


Figure 3.12. Enzymatic activities in the brain of mice at 7 days of bTBI. (A) represents Catalase Concentration. (B) represents NO Concentration, (C) represents SOD measurements. (D) represents GSH Concentration. (E) represents LPO Concentration. Values are expressed as mean ± SEM (n=3). $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$, ns: not significant.

3.10. Comparative analysis of FTIR Spectra

3.9.1 FTIR analysis of brain homogenate :

FTIR analysis was conducted on brain homogenate samples from female and male mice at 7 days post- traumatic brain injury (TBI) and 14 days post-TBI to identify changes in biochemical composition. Comparative FTIR analysis of brain homogenate at 7 days post-TBI revealed significant changes in the peak

intensities and positions in both bTBI males and females. These alterations indicate the presence of oxidative stress, protein damage, and changes in the total lipid content. However, at 14 days post-TBI, no differences were observed in the composition of brain homogenate compared to the control group, indicating potential recovery or stabilization of the biochemical changes.

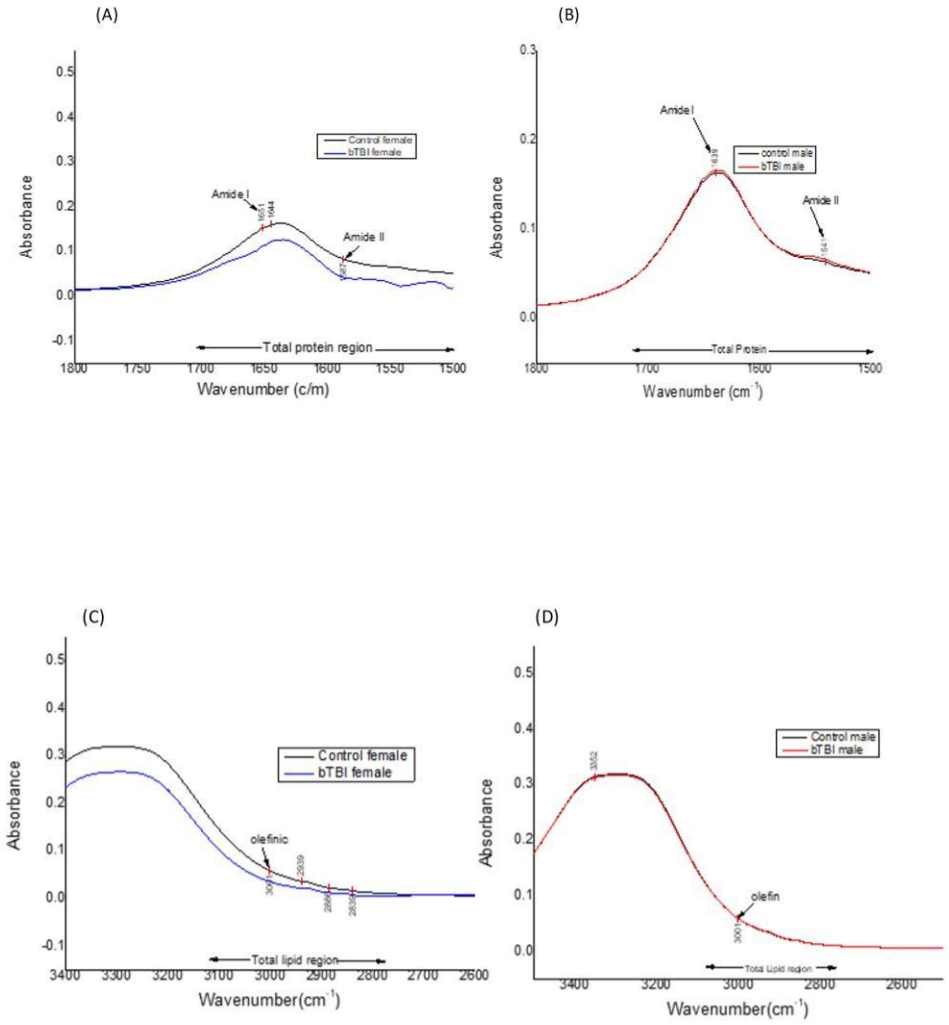


Figure 3.13. Representative spectra of brain homogenate at 7d post bTBI. (A) represents waveforms for protein (1700– 1500 cm-^{1}) region of interest. (B) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (C) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest. (D) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest.

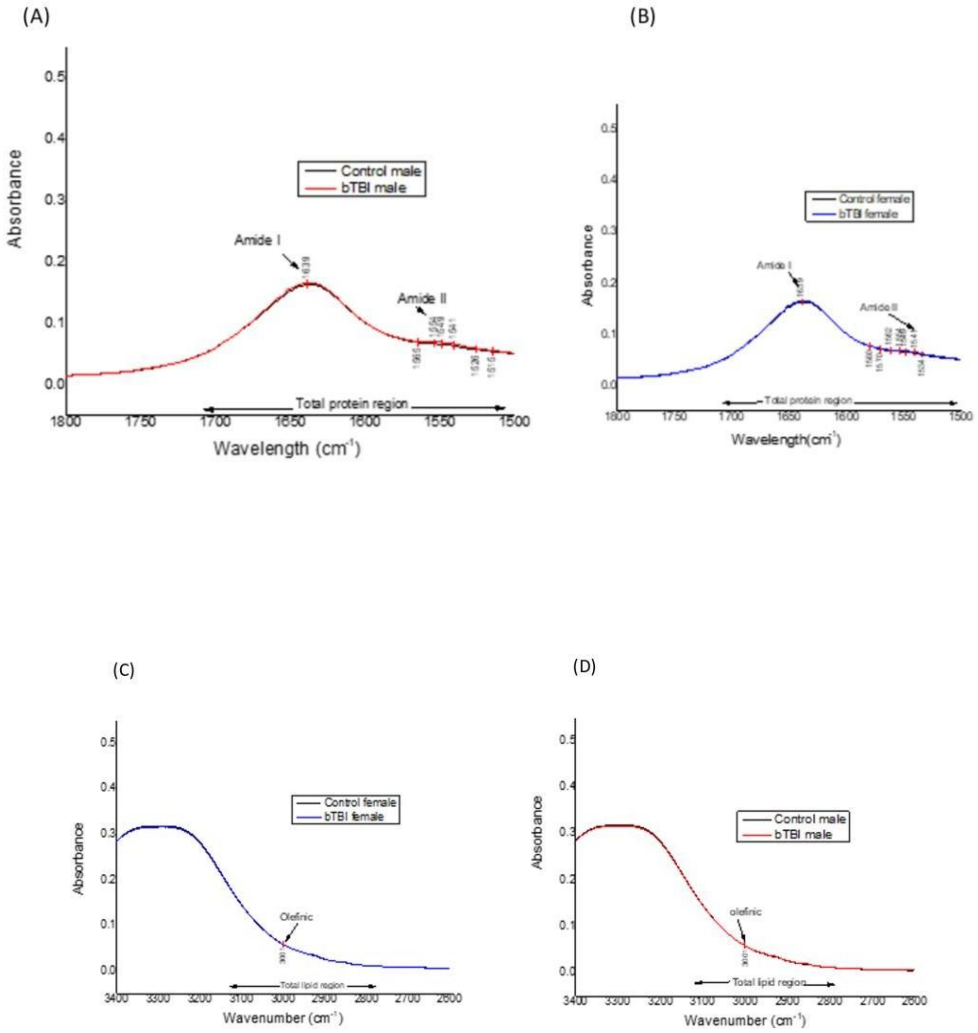


Figure 3.14. Representative spectra of brain homogenate at 14d post bTBI. (A) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (B) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (C) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest. (D) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest.

3.9.2. FTIR analysis of liver homogenate :

FTIR analysis was conducted on liver homogenate samples from female and male mice at 7 days post- traumatic brain injury (TBI) and 14 days post-TBI to identify changes in biochemical composition. Comparative FTIR analysis of liver homogenate at 7 days post-traumatic brain injury (TBI) revealed significant changes in the peak intensities and positions in both bTBI males and females. These alterations indicate the presence of oxidative stress, protein damage, and changes in the total lipid content. However, at 14

days post-TBI, no differences were observed in the composition of liver homogenate compared to the control group, indicating potential recovery or stabilization of the biochemical changes.

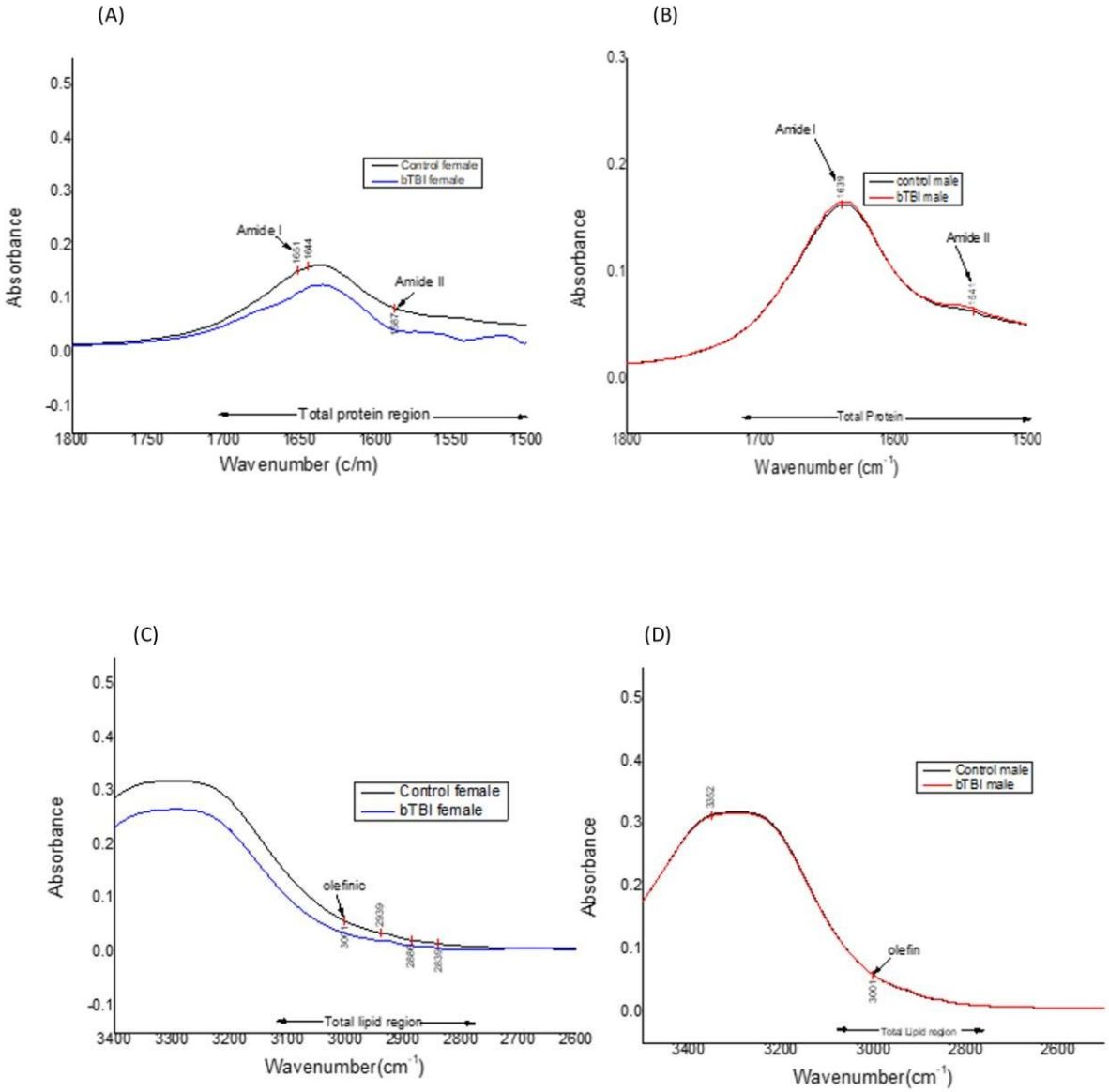


Figure 3.15. Representative spectra of liver homogenate at 7d post bTBI. (A) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (B) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (C) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest. (D) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest.

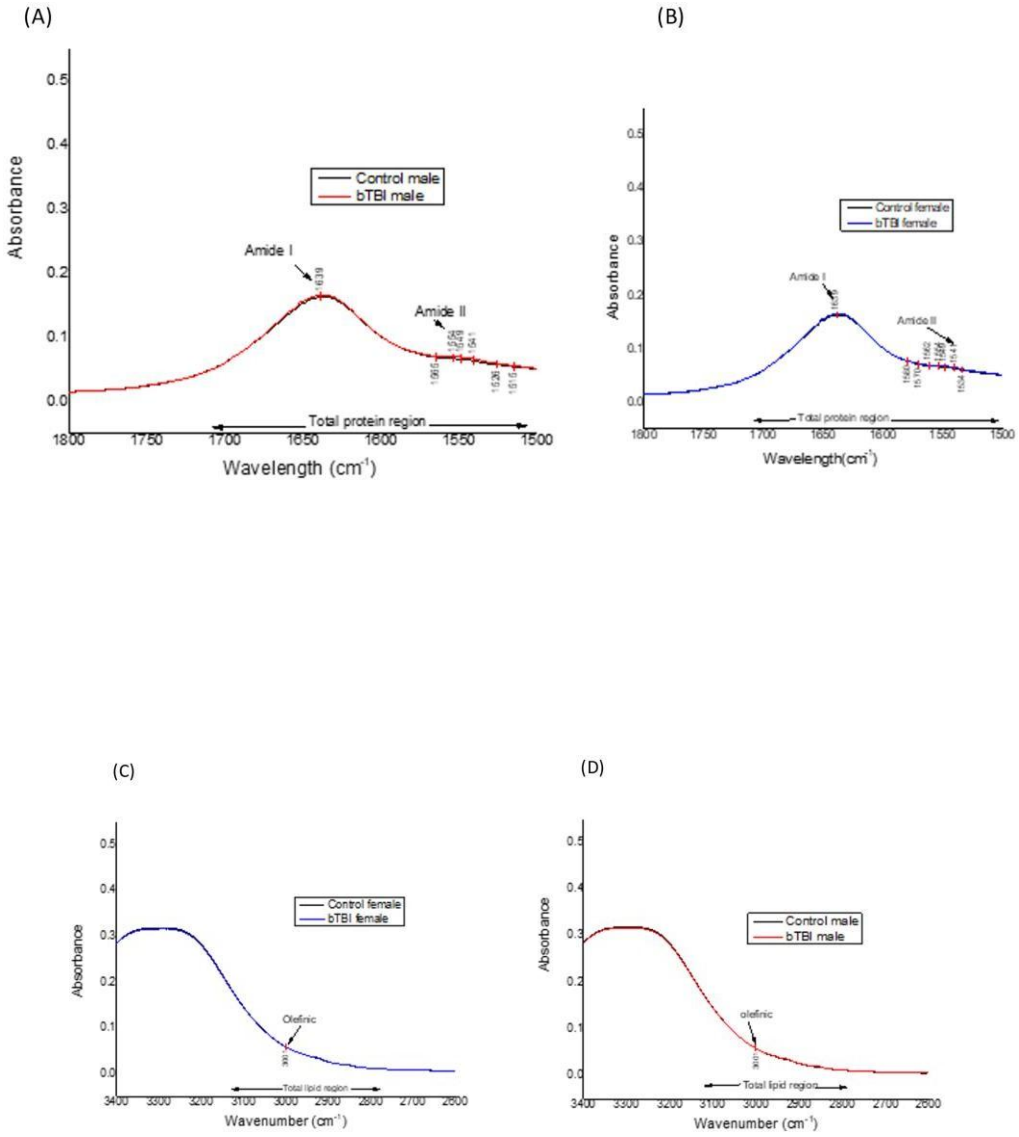


Figure 3.16. Representative spectra of liver homogenate at 14d post bTBI. (A) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (B) represents waveforms for protein (1700 – 1500 cm-^{1}) region of interest. (C) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest. (D) represents waveforms for lipid (3100 – 2800cm-^{1}) region of interest.

4. DISCUSSION

In the present study, we utilized a comprehensive analysis of various parameters, including the Neurological Severity Score (NSS), behavioral tests (Open Field, Beam Walk, Elevated Plus Maze), blood glucose, blood parameters, body weight, TTC staining, brain edema, antioxidant enzyme assays, Reactive Oxygen Species (ROS) levels, and FTIR spectroscopy to authenticate a bTBI model. Previous research has extensively studied the behavioral, histological, and molecular alterations associated with TBI (de Freitas Cardoso et al., 2019; Rabinowitz & Levin, 2014; Rakib, Al-Saad, Ahmed, et al., 2021). However, knowledge of the biochemical and spectroscopic changes that occur in the brain after blast TBI is limited.

The NSS and behavioral tests have been widely used to assess the neurological deficits and behavioral changes associated with TBI. The findings of the current study demonstrated a significant increase in NSS score, indicating mild injury. This finding is consistent with previous studies that have shown higher NSS scores in TBI models (A. L. Du et al., 2013; Stelfa et al., 2022; Tsenter et al., 2008). Our observations of alterations in motor coordination, exploration, and anxiety-like behavior are in line with previous research on TBI-induced behavioral changes (Aravind et al., 2020; W. H. Cheng et al., 2019; Hausser et al., 2018; Leconte et al., 2020; Popovitz et al., 2019; Stelfa et al., 2022). (Leconte et al., 2020) reported similar impairments in motor coordination and exploration in a rodent model of TBI. (Impellizzeri et al., 2016) also found increased anxiety-like behavior in a rodent model after TBI.

TBI is known to perturb glucose homeostasis and metabolic processes. In our study, we observed alterations in blood glucose levels, indicating disruption in glucose regulation after blast TBI. The results of the current study were found consistent with the previous study (Lai et al., 2022). (Moro et al., 2013) reported decreased blood glucose levels in a experimental animal model, indicating impaired glucose regulation. Similarly, (Demers-Marcil & Coles, 2022) found alterations in glucose metabolism in TBI patients, highlighting the systemic effects of brain injury on glucose homeostasis.

The evaluation of complete blood counts in male and female mice following blast traumatic brain injury (bTBI) revealed significant alterations in blood parameters, highlighting the systemic effects of TBI on hematological profiles. The current study demonstrated that male mice with bTBI exhibited significantly elevated levels of white blood cells (WBCs) and red blood cells (RBCs) compared to the control group, along with significantly decreased hemoglobin (Hb) levels and platelet counts. Conversely, female mice with bTBI exhibited significantly decreased WBC counts, RBC counts, and Hb levels, along with elevated platelet counts compared to the control group. The abnormal body weight observed post-TBI in our study are consistent with the detrimental effects of brain injury on appetite regulation and overall metabolic status.

TTC staining allows visualization of viable brain tissue, while brain edema reflects the extent of tissue swelling (Başkaya et al., 2000; Yang et al., 2021). The findings of current study revealed significant areas of infarction (indicated by non-stained regions) in the brain following blast TBI, which is indicative of tissue damage. Previous studies have also utilized TTC staining to assess tissue damage in various TBI models. (Yang et al., 2021) used TTC staining to examine tissue viability and infarction in an animal model. Their results demonstrated non-stained areas in the injured brain regions, indicating tissue damage consistent with findings.The presence of brain

edema observed in current study further supports the occurrence of tissue swelling, vascular dysfunction, and disrupted blood-brain barrier integrity in blast TBI. This aligns with prior research that has documented the association between brain edema and tissue damage in TBI (Dash et al., 2009; Donkin & Vink, 2010; Miller et al., 2021; Wang et al., 2011).

The enzymatic activities of catalase (CAT), superoxide dismutase (SOD), nitric oxide (NO), glutathione (GSH), and lipid peroxidation (LPO) were evaluated to assess the impact of bTBI on oxidative stress in the brain of mice. The results revealed significant alterations in enzymatic activities in the bTBI group compared to the control group. Previous research studies have also reported changes in antioxidant enzyme activities following TBI. (D. Du et al., 2021) demonstrated decreased CAT activity in a rat model of TBI, consistent with current findings. Similarly, (Yavtushenko et al., 2020) reported reduced SOD activity after TBI, aligning with the decreased SOD activity observed in the bTBI groups in current study. Elevated levels of nitric oxide (NO) in male mice with TBI, as observed in our study, are in line with the work of (Yin et al., 2021), who reported increased NO production in a chronic mild stress in male mice model . Furthermore, the decrease in glutathione (GSH) levels in both male and female mice with TBI aligns with previous study showing depleted GSH levels after TBI (Ignowski et al., 2018). Interestingly, while the current study did not find significant changes in lipid peroxidation (LPO) activity between the TBI and control groups, other studies have reported increased LPO activity after TBI (Yavtushenko et al., 2020).This discrepancy may be attributed to variations in the TBI models, severity, or time points assessed in different studies.The results also revealed a significant increase in ROS levels in the bTBI group compared to the control group, indicating an imbalance between ROS generation and antioxidant defense mechanisms. Previous research studies have consistently reported elevated ROS levels following TBI, supporting the current findings. (Kabu et al., 2015) demonstrated increased ROS production in an animal model of TBI, which corresponds to the oxidative stress observed in the study. Similarly, (Chen et al., 2019) reported elevated ROS levels in TBI patients, further corroborating the impact of TBI on oxidative stress in the brain.

In our study, FTIR spectroscopy analysis provided valuable insights into the biochemical alterations occurring in these tissues after bTBI. Changes in the spectral peaks corresponding to lipids and proteins indicated variations in their composition, secondary structure, and molecular interactions. Previous research studies have also utilized FTIR spectroscopy to investigate molecular changes associated with TBI. (Rakib, Al-Saad, Ahmed, et al., 2021) employed FTIR spectroscopy to analyze changes in brain tissue composition and structure after acute TBI. Their findings revealed alterations in lipid and protein content, supporting our observations of changes in spectral peaks corresponding to lipids and proteins. The variations in lipid composition and structure observed through FTIR spectroscopy are in line with the work of (Zhang et al., 2017), who reported changes in lipid content and organization in a traumatic axonal injury model.

4.4 Study Limitations and Future Directions

While this study provides important insights into the biochemical and spectroscopic changes that occur following bTBI, there are several limitations to consider. First, the use of a single animal model (Balb/c mice) limits the generalizability of the findings to other species, including humans. Future studies could explore the translational potential of these findings by validating the results in other animal models and ultimately in clinical settings. Additionally, the study focused on a relatively short post-injury time frame. Long-term studies are necessary to understand the chronic effects of bTBI and to evaluate the lasting biochemical and structural alterations that may persist beyond the acute injury phase. Moreover, while FTIR spectroscopy provided

valuable insights into molecular changes, it did not offer information on the spatial distribution of these alterations within the tissues. Combining FTIR with other imaging techniques, such as MRI or PET, could provide a more comprehensive picture of both the biochemical and structural impacts of bTBI. Future research should focus on exploring potential therapeutic interventions, such as antioxidant supplementation, and investigate their effectiveness in reversing or preventing the molecular changes observed in this study.

5. CONCLUSION

Summary

This study provides a comprehensive analysis of the biochemical and spectroscopic alterations that occur following blast traumatic brain injury (bTBI) in a mouse model. The findings reveal significant oxidative stress, as indicated by elevated levels of reactive oxygen species (ROS) and reduced antioxidant enzyme activity (such as catalase and superoxide dismutase). Additionally, FTIR spectroscopy identified molecular changes in both brain and liver tissues, with distinct shifts in protein and lipid bands, highlighting disruptions in cellular structure and function. These molecular alterations are consistent with the pathophysiology of bTBI, emphasizing the role of oxidative damage and inflammation in the injury process.

Clinical Relevance

The study on biochemical and spectroscopic characterization of mice brain after bTBI is highly significant. It helps in the identification of specific biochemical and molecular alterations associated with bTBI, aiding in a deeper understanding of the bTBI pathophysiology. This knowledge is vital for developing targeted therapeutic interventions and improving diagnostic techniques. Furthermore, the study contributes to the overall advancement of scientific knowledge in the field of bTBI research, potentially leading to improved outcomes for individuals affected by this condition. Future studies are needed to validate these findings in clinical populations and to explore the potential of FTIR as a routine diagnostic tool in TBI management.

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