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Neuroprotective effects of honey against traumatic brain injury-induced anxiety and motor function impairment in Wistar rats

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ABSTRACT: Traumatic brain injury (TBI) poses a significant risk to neurological function, primarily attributable to oxidative stress. Our hypothesis suggests that honey, renowned for its high phenol and flavonoid content, exerts neuroprotective effects by mitigating oxidative stress. This study aims to assess honey's potential as an innovative therapeutic agent in a TBI rat model, primarily focusing on its impact on behaviour, lipid peroxidation, and antioxidant enzyme activity. A total of twenty adult male Wistar rats were randomly divided into four groups: Group A (control), Group B (honey-treated), Group C (TBI-induced), and Group D (honey-treated TBI). We conducted comprehensive assessments using the Rotarod and Elevated Plus Maze tests to evaluate behaviour. Additionally, biochemical evaluations included quantifying lipid peroxidation levels and conducting a detailed analysis of antioxidant enzyme status, specifically emphasising the activity of glutathione (GSH) and catalase (CAT). Our findings indicated that the TBI model rats displayed impaired motor coordination, heightened anxiety-like behaviour, and elevated lipid peroxidation levels. Intriguingly, the honey treatment effectively reversed these behavioural deficits while concurrently reducing lipid peroxidation. Notably, honey treatment significantly augmented the activity of antioxidant enzymes (CAT and GSH); there was a significant increase in CAT activity compared to GSH activity in the treated TBI model. This study supports our hypothesis, demonstrating that honey is a potent neuroprotective agent that counteracts TBI-induced oxidative stress. Our investigation elucidates honey's capacity to alleviate neurobehavioral impairments and mitigate oxidative damage within a TBI model. These results underscore honey's significance as an innovative and promising therapeutic approach in TBI management, emphasizing its potential to enhance neurological outcomes and improve the overall well-being of individuals affected by TBI.

Keywords: Anxiety; Antioxidant; Honey; Traumatic brain injury; Motor function

1.0 INTRODUCTION

Traumatic brain injury (TBI) stands as a formidable challenge in contemporary healthcare, representing the foremost cause of enduring neurological impairments and mortality, with a particularly pronounced impact on individuals under the age of 45 (Chou et al., 2017; Lecca et al., 2019). Over the past decade, several animal models, including the controlled cortical impact (CCI) model by Khan et al. (2011), the weight drop model for mild TBI by Becker et al. (2018), and Xu et al. (2018), have been utilised to investigate various aspects of TBI pathophysiology (Johnson et al., 2015). A penetrating brain injury is a severe form of TBI (Solumsmoen et al., 2018) due to the brain's high oxygen consumption rate, it is highly vulnerable to oxidative stress (Mojtahedzadeh et al., 2014; Rodriguez-Rodriguez et al., 2014). Cerebral hypoxia and ischemia often occur secondary to TBI, resulting in impaired cerebral blood flow that leads to increased oxidative stress, neuroinflammation, excitotoxicity, and altered neural circuits. All these factors contribute to the pathogenesis of TBI, leading to impaired behavioural functions (Quillinan et al., 2016).

Natural antioxidants have been documented to remove oxidative stress inducers, particularly reactive oxygen species (ROS), thereby preventing lipid peroxidation and inflammatory responses caused by ROS production, hence averting brain oxidative damage (Huang et al., 2020). Currently, TBI medical management is mainly supportive, as no pharmacological therapies have demonstrated improvement in neurological outcomes (Corps et al., 2015; Gruenbaum et al., 2016). Therefore, there is a need to re-evaluate and understand the pathophysiology of TBI so that effective treatment and novel drug development can be made available for future interventions (O'leary & Nichol, 2018).

Honey is a natural food product made from the nectar of flowers by worker honeybees. It is a sweet, viscous substance with a strong antioxidant, as documented by Ali and Hendawy (2018a) and Ali and Kunugi (2019). Honey has various therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, and cardioprotective properties, and has been used to treat neurological disorders, fertility disorders, and wound healing, among other things (Ahmad et al., 2017; Ali & Hendawy, 2018b; Ali & Kunugi, 2019; Erejuwa et al., 2012; Rao et al., 2016). The therapeutic benefits of honey are linked to its phytochemicals, such as phenols, peptides, enzymes, minerals, and vitamins (Eteraf-Oskouei et al., 2013).

Oral ingestion of honey has the potential to enhance the endogenous antioxidant systems, including catalases and reduced glutathione (GSH) (Nathan & Cunningham-Bussel, 2013), thus mitigating TBI-induced ROS generation (Guo et al., 2013) and the subsequent neurodegeneration and behavioural deficits associated with TBI (Abdul-Muneer et al., 2015).

Oxidative stress has been shown to exacerbate neurovascular inflammation, blood-brain barrier (BBB) dysfunction, and neuronal cell death (Kramer et al., 2017; McKee & Lukens, 2016). Free radical production during oxidative stress produces protein oxidation, nitration, lipid peroxidation, and DNA damage (Guo et al., 2013). Studies have shown that the antioxidant capacity of honey is dependent not only on the presence of total phenolic compounds but also on the presence of flavonoids, which play an important role in ameliorating oxidative stress (Can et al., 2015; Flores et al., 2015).

TBI has been reported to cause disruptions in motor ability, alertness, balance, and general behaviour (Huang et al., 2016), all associated with oxidative stress. We aim to investigate the potential therapeutic role of honey, a natural antioxidant, in mitigating established neurological and behavioural deficits associated with TBI. Furthermore, our study seeks to comprehensively assess changes in malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) levels in response to honey treatment. Finally, we intend to evaluate the motor function and anxiety-like behaviour by utilising the rotarod test and the elevated plus maze, respectively. These objectives collectively form the framework for our research, aiming to shed light on novel interventions in managing TBI and ultimately improve neurological outcomes for affected individuals.

The significance of our study lies in its innovative approach, one that harnesses the therapeutic potential of honey to alleviate the debilitating consequences of TBI. Ultimately, we aspire to enhance neurological outcomes and overall patient well-being, building upon the foundation of previous research in this critical field.

2.0 MATERIALS AND METHODS

2.1 Experimental animals

The study employed twenty adult healthy Wistar male rats with an average weight of 120 ± 20 g obtained from the National Veterinary Research Institute (NVRI) in Vom-Jos, Plateau State, Nigeria. Before experimentation, the rats were allowed to acclimatise for two weeks. All animal care and behavioural testing procedures were conducted following the guidelines for the care and use of animals in research (National Research Council, 2011). The departmental research ethics committee approved this study, and the university ethics committee followed its guidelines. The rats were housed in well-ventilated metallic cages and were fed with rat-pelleted feed obtained from Vital Feeds Ltd. Nyanya, Nasarawa State, Nigeria, with water available *ad libitum*. They were maintained in standard pathogen-free (SPF) laboratory conditions, including a 12-hour light and 12-hour dark cycle, a temperature of 37 ± 2°C, and a relative humidity of 60 ± 5%. The study was conducted in the animal house of the Anatomy Department at Bingham University Karu, Nasarawa State, Nigeria.

2.2 Honey procurement and dosage administration for therapeutic use

Honey produced by worker honeybees was purchased from Azez Pharmaceutical Store in Nasarawa State, Nigeria. The dosage of honey used in the study was 0.5 mL/kg, as per the method outlined by Oyekunle et al. (2010). One tablespoon of honey is equivalent to 21 grams (21000 mg). One millilitre of honey equals 1440 milligrams. Hence, 0.5 mL/kg of honey is equivalent to 720 mg of honey per kg of a rat's body weight.

2.3 Experimental design

The total duration of experiment was two weeks (14 days). The experimental animals were randomly divided into four groups (n=5) as follows:

- Group A: Control group
- Group B: Received 0.5 mL/kg of honey orally once daily, for 14 days.
- Group C: Weight drop model of mild TBI
- Group D: Weight drop model of mild TBI, followed by 14 days of oral treatment with 0.5 mL/kg of honey; the treatment was given once daily.

2.4 Model of traumatic brain injury and induction procedure

The surgical area (scalp) was cleaned using methylated spirit. For surgical manipulations, including weight drop TBI modelling, animals were anaesthetised using chloroform. An incision measuring 1.5 cm was made on the midline of the scalp to perform the craniotomy. A tube-shaped metal object weighing approximately 400 g was then dropped from a height of 1 m, and guided to impact the left anterior frontal area, causing the desired TBI. To act as a shock absorber during the procedure, foam measuring about 10 cm thick was placed beneath the animals' heads. The craniotomy was conducted with a precise focus on the frontal lobe. The bregma was used as a landmark to determine the craniotomy coordinates accurately. Following the procedure, the craniotomy and skin were meticulously sutured, and the wound was cleaned with a 10% povidone-iodine solution (w/v, Betadine) to prevent the occurrence of infection, as suggested by Pandey et al. (2009) and Xu et al. (2018). It should be noted that craniotomy can contribute to brain damage in our TBI model, as reported by Cole et al. (2011). Any animal that experienced excessive bleeding due to disruption of the dura during the experiment was excluded from the study. The sutured and cleaned rats were kept in a clean, sterile cage, closely monitoring the wounds and the healing process. Once they exhibited normal walking and grooming behaviour, the rats were returned to their home cage after recovery. The recovery time post-induction of TBI was determined based on careful observation. Animals were considered adequately recovered within 3 to 4 days post-TBI induction when they displayed increased activity levels, and a gradual healing process was observed at the surgical site (**Figure 1**).

In the initial pilot study, a notable mortality rate was observed, with 3 out of 5 animals in one group succumbing to infection and haemorrhage. To address this concern and enhance experimental integrity, the study was repeated with meticulous attention to surgical techniques to minimise infection risk and reduce bleeding. Additionally, measures were taken to ensure the preservation of the dura mater. As part of the exclusion criteria, animals with severed dura were excluded from the experiment, excluding 2 animals.

2.5 Behavioural testing

As reported by McKee and Lukens (2016) and Kramer et al. (2017), neurodegeneration induced by TBI is associated with behavioural deficits, which underscores the need to assess motor coordination function using the rotarod test (Wagner et al., 2019) and anxiety-like behaviour using the elevated plus maze (Biedermann et al., 2017).

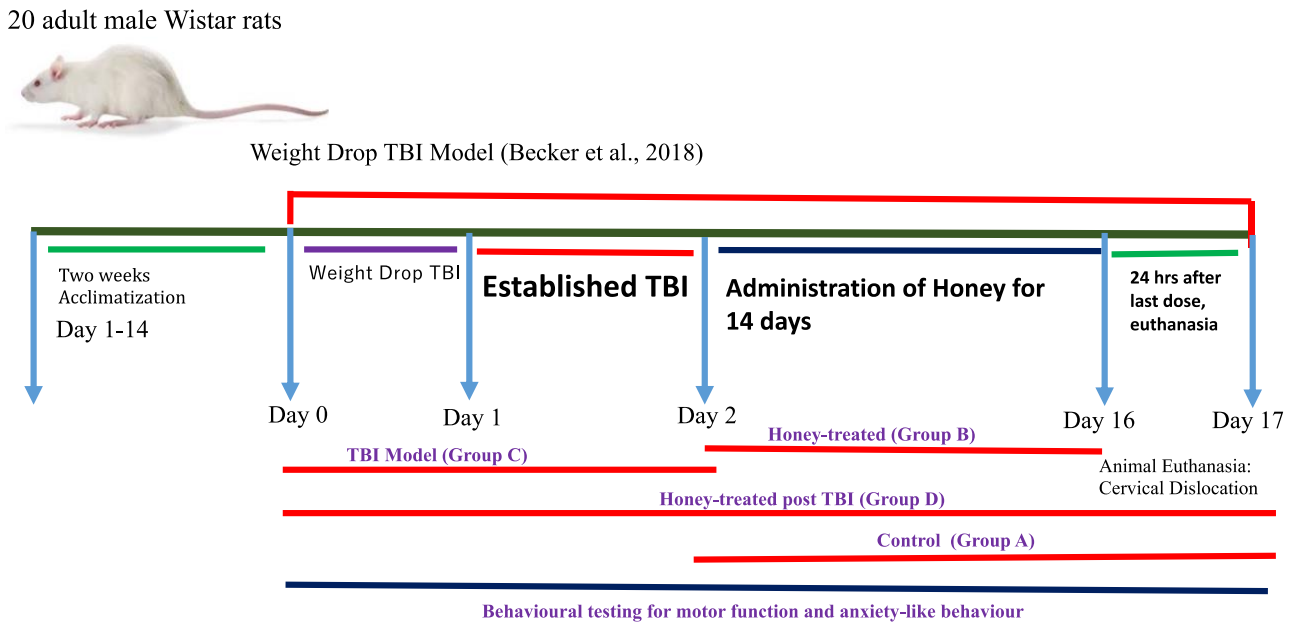

Figure 1: This schematic diagram illustrates the comprehensive experimental design, timeline, and key events for the study. The diagram provides a visual representation of the progression of the experiment, including the induction of traumatic brain injury (TBI), honey treatment, behavioural assessments, and sacrifice time points for each experimental group. Arrows indicate the direction of the experiment, with different line lengths depicting variations in group sizes. The term "established TBI" signifies when the TBI induction was completed. The figure aids in understanding the temporal sequence of events and the allocation of animals into distinct groups for data collection and analysis.

Rotarod test

To evaluate motor coordination, balance, and motor learning, the animals were subjected to the rotarod test, as recommended by Morales et al. (2018) and Wagner et al. (2019). The animals were transferred to the experimental room 20 minutes before the commencement of the test to allow for muscular recovery. They were held by the tail and positioned on the rotating rod, facing away from the direction of rotation (Wagner et al., 2019). Each trial was conducted three times (Morales et al., 2018), and the number of revolutions per second (rps) was recorded for each trial. If a rat failed to grip the rod within 10 seconds, they were assigned a score of 4 revolutions per minute (rpm). The mean number of revolutions per second was then calculated, following the approach recommended by McKee and Robinson (2014).

Elevated plus maze (EPM)

The Elevated Plus Maze (EPM) test is a widely recognised preclinical method employed to assess anxiety-like behaviour in rats (Biedermann et al., 2017). In this test, rodents tend to avoid open arms and prefer closed arms, which indicates anxiety and reduced risk tolerance (Biedermann et al., 2017; Walf & Frye, 2013). The EPM apparatus used in our study was configured in the shape of a plus sign (+) and consisted of two open arms (25 x 5 x 0.5 cm) intersecting with two closed arms (25 x 5 x 16 cm), all connected by a central platform (5 x 5 x 0.5 cm) (Albani et al., 2015; Wang et al., 2018). This maze was constructed from plywood, ensuring a stable and controlled environment for testing.

To comprehensively assess the behavioural response of the rats in the elevated plus maze, we employed a video recording system to monitor and record their activities. Specifically, we recorded the number of entries into the closed arms and the time spent in the closed arms over a 5-minute observation period (Mijanur Rahman et al., 2014). These measurements provide valuable insights into the anxiety levels and risk avoidance behaviours of the rats, helping us evaluate the impact of honey treatment on anxiety-related parameters.

2.6 Blood collection for serum enzyme assays

Blood samples for serum biochemical analysis were collected via cardiac puncture with a clean capillary tube and transferred to labelled plain specimen bottles. Serum was obtained by centrifuging the sample at 3,000 rpm for 10 minutes, following the method described by Huang et al. (2020). The serum was then aliquoted into cuvettes and assayed for enzyme activity using a spectrophotometer and enzyme-specific commercial assay kits.

Estimation of Malondialdehyde (MDA) activity

MDA is a biomarker for lipid peroxidation (Abdul-Muneer et al., 2015). The plasma level of MDA was assessed using a commercial kit and spectrophotometer (Huang et al., 2020). MDA activity was quantified using the commercial kit (NWKMDA01, Northwest Life Sciences Specialties, United States). This kit was selected due to its established reliability in assessing lipid peroxidation.

Estimation of antioxidant enzymes CAT and GSH

Antioxidant enzymes, such as catalase (CAT) and glutathione (GSH), are endogenous antioxidants that provide primary protection against oxidative stress (Huang et al., 2020; Malkoç et al., 2020). The activities of CAT and GSH enzymes in the serum were measured using a spectrophotometer (Huang et al., 2020), following the manufacturer's protocol supplied with the commercial kit (Northwest Life Sciences Specialties, United States).

2.7 Statistical analysis

Statistical analysis was performed according to the method described by Jatana et al. (2006) using GraphPad Prism 8.0 software (GraphPad Software Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's posthoc test was used to determine the level of statistical significance between the groups, with a threshold set at $p<0.05$. All data was presented as mean ± standard deviation (SD). The Tukey posthoc test was used for multiple comparisons, and p values < 0.05 were considered significant.

3.0 RESULTS

3.1 Honey treatment improves antioxidant enzymes (CAT and GSH) status in TBI

TBI decreases circulating levels of CAT and GSH, which is reversed by treatment with honey. Honey treatment group (B) led to increased activities of GSH and CAT compared to the control group and TBI model at a statistically significant level of $p<0.001$. The TBI model exhibited a decrease in GSH and CAT activities compared to the control group at a statistically significant level of $p<0.001$. In the honey-treated TBI model (Group D), there was a significant increase in glutathione activity compared to the TBI model at a statistically significant level of $p<0.001$. The honey-treated TBI model (Group D) also showed increased CAT activity compared to the TBI model at a statistically significant level of $p<0.001$. The activity of glutathione increased significantly in the honey-treated group compared to the control group ($p<0.001$). The TBI model group exhibited a significant decrease in glutathione activity compared to the control group ($p<0.001$). In contrast, the honey-treated TBI model group showed a significant increase in glutathione activity compared to the TBI model group ($p<0.001$). Moreover, the honey-treated group showed a significant increase in glutathione activity compared to the honey-treated TBI model group ($p<0.05$). The activity of CAT increased significantly in the honey-treated group compared to the control group ($p<0.001$), and the honey-treated TBI model group showed a significant increase in CAT activity compared to the TBI model group ($p<0.001$) (**Figure 2**).

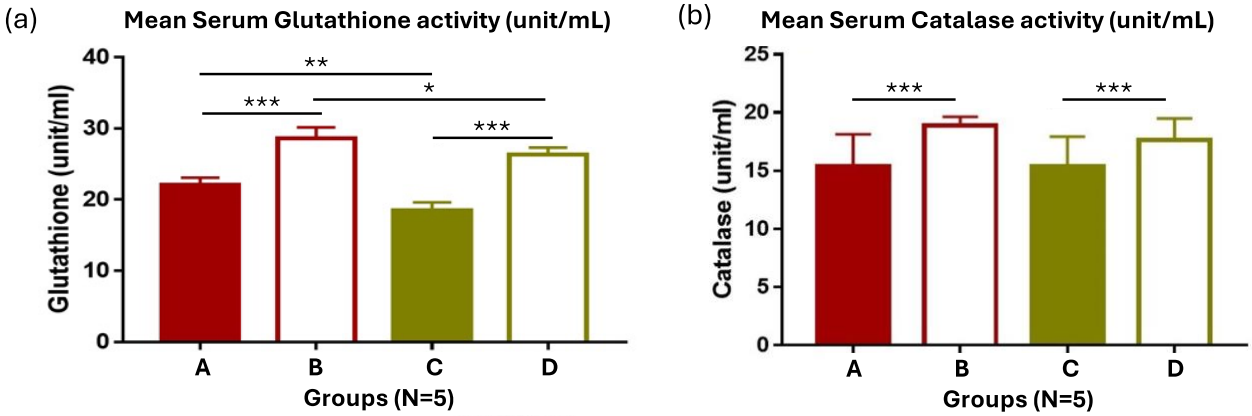

Figure 2: The figures illustrate the mean activity levels of antioxidant enzymes: (a) Glutathione (GSH) and (b) Catalase (CAT) in the experimental Wistar rats. Data were analyzed using one-way ANOVA and presented as mean ± SD, with Tukey's post hoc test applied for statistical significance. Significance levels are $p<0.05$ and $p<0.001$ (N=5). Group legend: Group A (Control), Group B (Rats receiving 0.5 ml/kg body weight of honey for 14 days), Group C (Weight drop model of mild TBI), and Group D (Weight drop model of mild TBI followed by 14 days of oral honey treatment). Statistical significance was determined by one-way ANOVA followed by Tukey post hoc test, with *$p<0.05$ and ***$p<0.001$ considered statistically significant for N=5.

3.2 TBI-induced lipid peroxidation was reversed by honey treatment

A significant increase in MDA level was associated with elevated lipid peroxidation in the TBI group compared to the control, honey, and honey-treated TBI model groups ($p<0.001$). The honey-treated TBI model group had significantly declined MDA activity compared to the TBI model group ($p<0.001$) (**Figure 3**).

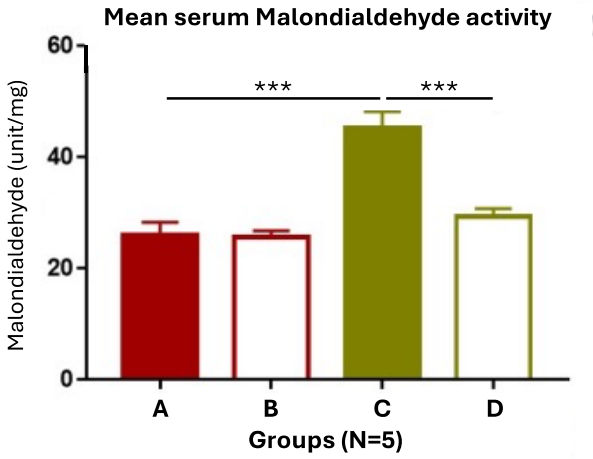

Figure 3: Average lipid peroxidation levels measured by malondialdehyde biomarker in experimental Wistar rat groups. Data analyzed using One-way ANOVA followed by Tukey post hoc test (N=5, significance at $p<0.05$ and $p<0.001$). Honey treatment significantly decreased MDA levels in both control and TBI groups compared to the untreated TBI model group (***$p<0.001$).

3.3 Honey attenuated TBI-induced motor dysfunction

Compared to the TBI group, the control group demonstrated a significant decrease in revolutions and time spent on the rotarod ($p<0.001$). Moreover, the control group had increased revolutions and time spent compared to the honey-treated group. Conversely, the honey-treated TBI model group exhibited a significant increase in the number of revolutions and time (seconds) spent on the rotarod compared to the TBI model ($p<0.001$). There was an increase in the time spent in the close arm of the elevated plus maze, as observed in the TBI model compared to the honey-treated, honey-treated TBI model and the control groups. Also, the number of arm entries decreased in the TBI model compared to the honey-treated TBI model. This indicates poor motor function, attributed to TBI induction, as demonstrated on the rotarod test. Furthermore, the rotarod test for locomotion shows that the TBI model declined the number of revolutions and time spent on the rotarod. However, the honey-treated TBI group had significantly increased number of revolutions and time spent on the rotarod (**Figure 4**).

3.4 Honey reverses TBI-induced anxiety in elevated plus-maze test

The TBI model group spent significantly more time in the closed arm of the EPM compared to the control, honey, and honey-treated TBI model groups ($p < 0.001$).

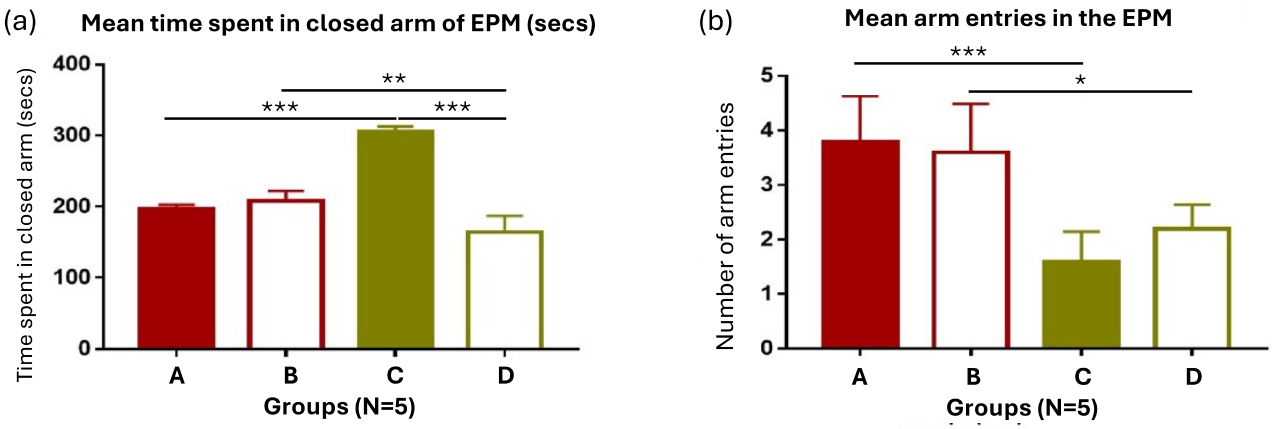

Figure 4: Elevated Plus Maze analysis of experimental Wistar rats showing mean time spent in closed arms (a) and number of arm entries (b). Data analyzed using One-Way ANOVA with Tukey post hoc test (N=5, significance at $p<0.05$ and $p<0.001$). Group legend: Group A (Control), Group B (Rats receiving 0.5 ml/kg body weight of honey for 14 days), Group C (Weight drop model of mild TBI), and Group D (Weight drop model of mild TBI followed by 14 days of oral honey treatment). Honey treatment significantly altered anxiety-like behavior, with notable changes in closed arm time and arm entry patterns compared to the TBI model group.

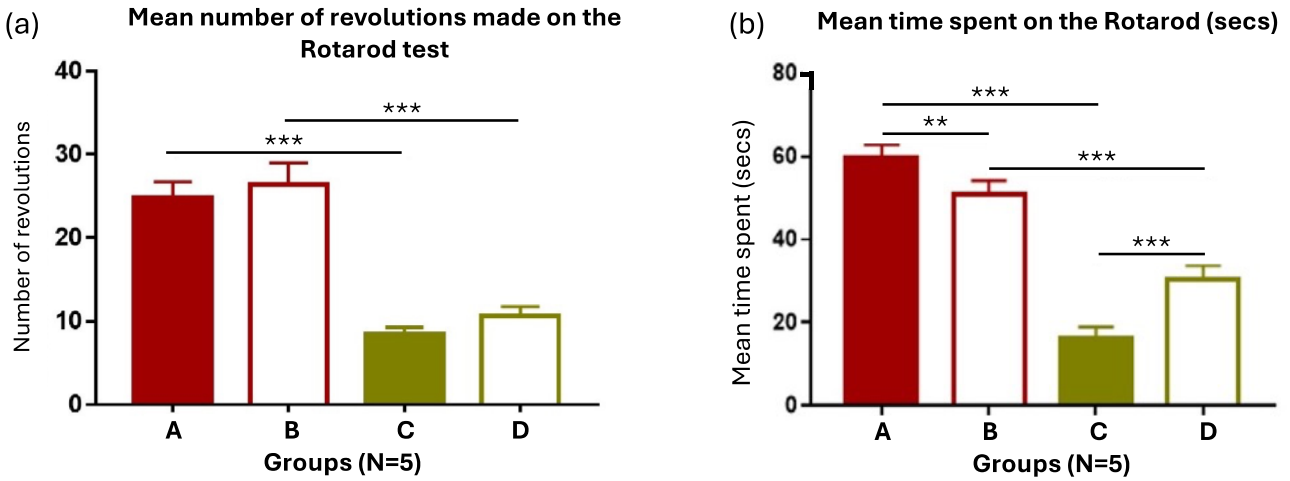

Figure 5: Rotarod performance analysis of experimental Wistar rats, measuring motor coordination and balance through mean time spent and number of revolutions. Data analyzed using One-way ANOVA with Tukey's post hoc test (N=5, significance at $p<0.05$ and $p<0.001$). Experimental groups include Group A (Control), Group B (Rats receiving 0.5 ml/kg body weight of honey for 14 days), Group C (Weight drop model of mild TBI), and Group D (Weight drop model of mild TBI followed by 14 days of oral honey treatment). Results demonstrate significant differences in motor performance, with the honey-treated TBI group showing improved rotarod performance compared to the untreated TBI model group (**$p < 0.05$ and ***$p < 0.001$).

4.0 DISCUSSION

TBI is a neurodegenerative disorder with debilitating neurological deficits. There is a need to study and report new and accessible therapies to combat brain injury, which triggers oxidative stress-mediated tissue nerve and neuron damage.

In our previous report, we found that TBI causes chromatolysis and necrosis, resulting in a decline in superoxide dismutase (SOD) activity and an elevation in lipid peroxidation (Dennis et al., 2022), ultimately leading to the loss of cortical neurons in the prefrontal cortex, thinning of axons and dendrites, and a decrease in synaptic density which correlates with various studies conducted by Chou et al. (2017), Mahboob et al. (2016), and Assefa et al. (2018).

In this study, TBI caused a marked decline in antioxidant enzymes GSH and CAT, resulting in a corresponding increase in MDA activity, a biomarker for lipid peroxidation; this further supports the notion that honey supplementation can increase CAT and GSH levels in plasma, as previously suggested by Kramer et al. (2017). In our present study, honey aids in the cellular increase of antioxidant enzymes, which fights against the progression of lipid peroxidation and oxidative tissue damage (Dennis et al., 2022; Malkoç et al., 2020). This protects the neurons from damage by mobbing off ROS release due to TBI induction, as shown in **Figures 2a** and **2b**. This is supported by various studies that discovered honey administration can reverse TBI-mediated neuron damaging effects by an increase in endogenous antioxidants, such as CAT and GSH (Nathan and Cunningham-Bussel (2013), Malkoç et al. (2020), and Dennis et al. (2022)

Our findings showed that TBI results in a depletion of endogenous antioxidants compared to the honey-treated TBI model. This finding correlates with previous reports by Ansari et al. (2008b), which explained the role of oxidative stress in ROS-mediated neuronal cell death, and Becker et al. (2018), which highlighted the depletion of antioxidant enzymes in TBI models, leading to the accumulation of highly reactive free radicals or ROS, and resulting in the loss of neuron integrity and function of cell membranes, as discussed by Hasenan et al. (2017) and Dennis et al. (2022).

Oxidative stress and inflammation are closely related, leading to cell lipid peroxidation, which can be detected using MDA, a biomarker for TBI, as established in previous studies by Abdul-Muneer et al. (2015), Özevren et al. (2018), Huang et al. (2020), and Dennis et al. (2022). Our study shows that TBI elevates MDA levels (**Figure 3**), which decline upon treatment with honey. Honey supplementation in TBI significantly decreased lipid peroxidation in serum, with concomitant augmentation of antioxidants, as supported by Rao et al. (2016) and Gholami et al. (2017). This finding further supports the notion that increased levels of glutathione in the serum are associated with better outcomes in therapeutics for TBI, as reported by Wang et al. (2016)

and Du et al. (2016). Thus, a decline in MDA with elevated antioxidant levels can demonstrate positive outcomes for any drug used to manage TBI. There have been reports indicating that TBI may result in an imbalance in lipid peroxidation markers and antioxidant enzymes, leading to neurological deficits and neuron cell death (Dennis et al., 2022; Liang et al., 2018; Yang et al., 2016). Our present study demonstrates that TBI caused an imbalance between other classes of antioxidant enzymes studied, such as CAT and GSH having a decline and resulting in an increase in MDA activity, resulting in the disruption of the neuronal activity demonstrated via the behavioural deficits in rotarod test and elevated plus maze as seen in the decline in number of arm entries and time spent on the rotarod test.

To establish whether TBI can induce anxiety-like behaviour or mood-related disorders using the elevated plus-maze, we observed that the TBI model spent more time in the closed arm. However, this behaviour was reversed by honey treatment in our study, which correlates with previous findings by Akanmu et al. (2011) who suggested honey has anxiolytic potential. This effect is linked to the antioxidant contents of honey, which are rich in phenolic and flavonoid compounds that can attenuate intracellular oxidative damage associated with cellular necrosis in neurodegenerative diseases (Luchese et al., 2017).

Furthermore, to investigate the effects of TBI on motor function and anxiety-like behaviour, we conducted a study using rotarod and elevated plus-maze tests. Our results showed that TBI model rats experienced a significant decline in motor function, as demonstrated by decreased revolutions and time spent on the wheel during the rotarod test (**Figure 5**), and several arm entries made in the elevated plus-maze. However, this decline was reversed following treatment with honey for 14 days. This finding supports previous reports by Gholami et al. (2020) who suggested honey improves cognitive and mood-related behaviours associated with the loss of neural circuits and connections. Our recent report on how honey protects against TBI-induced neurodegeneration further supports honey-mediated neuron repair in TBI (Dennis et al., 2022).

Neurodegenerative disorders, neurological disorders, and mood disorders have all been associated with neuroinflammation and oxidative stress, which are marked by an elevation in lipid peroxidation, a decline in antioxidant enzymes, and neuronal cell death, leading to behavioural dysfunctions (Malkoç et al., 2020; Xu et al., 2018). According to Lecca et al. (2019) and Çetin and Deveci (2019), TBI pathogenesis affects neurobehavioral functions. It promotes inflammatory events that lead to neuronal dysfunction, characterised by a marked loss of synaptic density and cortical neurons. However, the brain can protect itself by producing numerous endogenous antioxidants (Huang et al., 2020).

Furthermore, TBI contributes to various mental health disorders, such as anxiety and depression (Al-Kader et al., 2022). Hence, we study the potential of honey in ameliorating TBI-induced anxiety-like disorders using the behavioural test paradigm elevated plus maze. The result demonstrated that TBI model rats spent more time in the close arm of the elevated plus maze (Kraeuter et al., 2019). In this study, TBI caused animals to spend more time in the closed arm of the elevated plus maze than the control and honey-treated group. This finding on TBI causing anxiety-like behaviour correlates with reports in various studies (Boyko et al., 2022; Fillippone et al., 2023; Popovitz et al., 2019). However, honey administration revised this anxiety-like behaviour as seen in a decline in the amount of time spent in the closed arm of the elevated plus maze (**Figure 4**). This potential of honey to improve neural function as a potential anxiolytic agent correlates with other reports on honey's potential to alleviate mood disorders as supported by reports (Ali & Hendawy, 2018b; Azman et al., 2019; Zakaria et al., 2022; Zamri et al., 2023).

It is important to mention that reports on honey alleviating mood disorders and other forms of neurological disorders, are associated with its neuron protective potential linked to the abundant form of diverse polyphenols with strong antioxidant potential (Fadzil et al., 2023; Iftikhar et al., 2022; Zulkifli et al., 2023) as shown in this present study. 8. TBI is a cerebral small vessel disease due to cerebral haemorrhage into the brain parenchyma or tissue (Che Mohd Nassir et al., 2022); hence, honey helps to protect the nervous tissue from oxidative tissue damage associated with cerebral haemorrhage. In this study, based on the aforementioned neuroprotective role of honey, the honey-treated TBI model had increased endogenous antioxidants CAT and GSH, which aids in protecting the neurons from oxidative tissue damage, that would result in neurocognitive deficits. Hence, in the honey-treated TBI, there were improved neurocognitive functions seen in an increase in motor function and alleviation of anxiety behaviour. This shows that honey helps to protect neuron activity and connection, as reported earlier in our previous study (Dennis et al., 2022), through its ability to avert neuronal cell death, increase

lipid peroxidation, and decline antioxidant enzymes due to ROS generation, which ultimately leads to injury to the axonal network, causing cognitive behavioural dysfunction (Chen et al., 2018; Yates et al., 2017).

TBI-induced neurobehavioral dysfunctions result from oxidative damage in the neurovascular unit. These pathological events are characterised by the continuous production of reactive oxidising species (ROS) and neuroinflammation. This leads to disruption of synaptogenesis on axons or myelin loss, altered tissue homeostasis, and, subsequently, neuronal cell death. These complications may trigger neurological disorders such as epilepsy, depression, anxiety, and dementia (Hampshire & Sharp, 2015; Karve et al., 2016).

However, honey has been found to possess neuroprotective properties due to its phytochemical constituents, such as phenols, flavonoids, minerals, and vitamins C and E. These constituents can neutralise ROS and prevent DNA damage, as has been shown in previous studies (Ciulu et al., 2016; Kramer et al., 2017). Our findings in this current study, suggested that honey has the potential to attenuate TBI-mediated neurobehavioral deficits by neutralizing ROS and preventing further neurovascular damage.

It is important to acknowledge certain limitations in our study. Firstly, the absence of a sham surgery control group in the current experiment is noted. While this limitation may introduce variability due to surgical and anaesthesia-related impacts in the control group, it is essential to emphasise that the primary focus of our study was on assessing the effects of honey treatment on TBI-induced behavioural and biochemical changes. Future studies incorporating a sham surgery control group will provide a more comprehensive evaluation of TBI-specific effects.

Furthermore, we maintained the laboratory temperature at 37 ± 2°C, following established guidelines for TBI research. While temperature variations can influence TBI outcomes, our adherence to the recommended temperature range aligns with best practices and standards in the field. The overall impact of this temperature regulation on our study results is considered minimal.

While we acknowledge the limitations mentioned above, it is essential to underscore the significant contribution of this study to the field of traumatic brain injury research. This study investigated the potential of honey to mitigate the negative consequences of traumatic brain injury. Our findings demonstrated that honey treatment in a TBI model increased antioxidant enzyme level (GSH and CAT) and decreased lipid peroxidation, indicating its potential to reduce oxidative stress. Additionally, honey treatment reversed TBI-induced motor dysfunction and anxiety-like behaviour in rats. These findings suggest that honey may act by scavenging free radicals and modulating antioxidant pathways, thereby protecting neurons from oxidative damage. Further research is warranted to explore the underlying mechanisms of honey's effects and its efficacy in different TBI models and treatment regimens. This study opens a promising avenue for developing novel therapeutic strategies to improve the lives of individuals affected by TBI.

5.0 CONCLUSIONS

Traumatic brain injury induces oxidative stress, characterized by elevated malondialdehyde levels and a decline in antioxidant enzyme activities. This oxidative damage can lead to impaired motor function and potentially contribute to the development of anxiety-like behaviors. Honey treatment has been shown to mitigate these effects by reducing lipid peroxidation and enhancing antioxidant enzyme activities, thereby potentially improving motor function and alleviating anxiety-related symptoms associated with TBI.

Author contributions: AEM, DD, and IC conceived the idea and designed the experiments; DD performed the experiments; AEM and DD analyzed the data; AEM and DD contributed reagents/materials/analysis tools; AEM and IC wrote the initial version of the paper; IC corrected and finalized the manuscript.

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