Quantitative Phytochemistry and Neuro-histological Effect of *Tamarindus indica* Fruit Pulp Aqueous Extract on Traumatic Brain Injury in Albino Rats


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**ABSTRACT**

*Tamarindus indica* has numerous therapeutic benefits. Its therapeutic effects have been reported in some neurodegenerative conditions like Alzheimer's disease in rats. This study evaluated quantitative phytochemistry and effect of tamarind fruit pulp extract in traumatic brain injury (TBI) - induced neurobehavioral and histological changes in rats. Six groups of seven rats were used for this study. Groups 1, 2, 3, and 4 were treated with tamarind fruit pulp extract at doses of 100, 200, 400, and 800 mg/kg, orally, after being induced with TBI. Rats in group 5 were traumatized but not treated (TNT), while rats in group 6 were not traumatized and were not treated (NTNT). The administration of treatment commenced 30 minutes after the occurrence of traumatic brain injury (TBI) and persisted for 21 consecutive days. The fruit was extracted using water, while HPLC quantified the phytochemicals. Neurological severity scores and novel object discrimination tests were carried out. The histological appearance of the brain tissue was also evaluated. The qualitative phytochemistry of tamarind fruit pulp extract contains alkaloids 17.72 mg/g, flavonoids 17.48 mg/g and tannin 10.35 mg/100g in the HPLC analysis. Tamarind fruit pulp extract improved neurological scores and memory function in the treated groups compared to group V, which did not show improvement. Histological results showed few lesions in the treated groups, while in the TNT group, massive and diffused lesions were observed. This study has shown that *Tamarindus indica* fruit contains neuro-therapeutic substances which may benefit TBI patients by improving their neurological and memory function.

**Keywords:** Albino Rats; Histology; HPLC; Neurological severity score (NSS); Phytochemistry; Traumatic Brain Injury (TBI); *Tamarindus indica*

**INTRODUCTION**

Brain injury is caused by an external mechanical force, perhaps resulting in temporary or permanent damage. The coexistence of cognitive, physical, and psychosocial impairments, along with a simultaneous decrease or alteration in the state of consciousness is presented (Segun et al., 2019). The global incidence of traumatic brain injury (TBI) in children is mostly concentrated in low- and middle-income countries (LMIC), which collectively represent 95% of this burden. (Appenteng et al., 2018). The impact of TBI in Nigeria is huge owing to the increased violence, accidents and stretched healthcare facilities. (Olabisi et al., 2021). In Nigeria, head injury was observed to be the most common among all injuries (Adeleye and Ogun, 2017). An incidence rate of 2710/100,000 per year has been reported in a tertiary hospital in south-east Nigeria (Emejulu et al., 2010). A prospective study in a tertiary hospital in Bauchi, northeast Nigeria, reported 537 cases in one year (Olabisi et al., 2021). One of the primary consequences of traumatic brain injury (TBI) is the substantial limitations that individuals endure even after the process of recovery. These deficits may encompass various impairments in cognitive processes, such as thinking and memory, motor skills, sensory perception (e.g., vision or hearing), or emotional regulation. These issues not only exert influence on individuals but also possess the potential to generate enduring consequences for families and communities. (Thurman and Guerrero, 1999). Memory impairment is reported as one of the most consistent and persistent cognitive deficits following TBI, with slower
recovery that may linger for years (Zec et al., 2001). Both primary and secondary injuries in TBI can cause memory deficits.

The pathophysiological mechanisms behind traumatic brain injury (TBI) encompass both primary and secondary damage processes. The initial mechanical impact during an injury is the primary cause of specific anatomical lesions in different parts of the brain (Dokin and Vink, 2010). The condition can be escalated by excessive discharge of excitatory amino acids, and inadequate perfusion has the potential to result in the malfunction of cellular ion pumps, hence triggering a cascade of events involving intracellular calcium and sodium influx. The presence of excessive calcium and salt might potentially lead to malfunction of the ion pump, as the cascade progresses, cellular death ensues, leading to the generation of free radicals. (Noppens et al., 2004; Bigler and Maxwell, 2011; Pandit et al., 2013).

The investigation of plants possessing antioxidative properties has garnered growing attention due to the significant roles played by antioxidative chemicals in the management and prevention of diseases associated with oxidative stress induced by free radicals. (Boligon et al., 2009). Natural products present promising prospects for mitigating the advancement and manifestations of neurodegenerative diseases. Notably, plants containing flavonoids, lignans, polyphenols, tannins, sterols, triterpenes, and alkaloids have garnered significant interest due to their demonstrated antioxidants, anti-inflammatory, anticholinesterase, and anti-amyloidogenic properties. Examples of such plants include Curcuma longa, Bacopa monnieri, Convolvulus pluricaulis, and Centella asiatica (Larit and Leon, 2023).

Tamarind exhibits a wide range of pharmacological activities, such as antioxidant, hypoglycaemic, antihyperlipidemic, antimicrobial, immunomodulatory, antivenom, antiplatelet, and anti-inflammatory activities (Sofowora, 1996; Elmaidomy et al., 2022). Chunglok et al., (2014) reported the higher content of polyphenol compounds and antioxidative compounds in Tamarindus indica seeds than other plant seeds which is believed to provide neuroprotection during neurodegeneration. Both Pumthong, (1999) and Tsuda et al., (1994) described the antioxidant activity of Tamarind extract invitro. Restoration of cognitive function post-TBI remains a challenge and requires more research to unravel possible therapeutic agents with memory-enhancing / restoring effects. The present work investigated the neurotherapeutic benefits of an extract derived from the fruit pulp of Tamarindus indica in rats with experimentally induced traumatic brain injury (TBI).

MATERIALS AND METHODS

Experimental Animals

This investigation involved the acquisition of forty-two (42) healthy albino rats with weights ranging from 150-200g. The rats were obtained from the Faculty of Veterinary Medicine animal house, University of Maiduguri, Nigeria. The rats were given a period of three weeks to adapt to the environmental circumstances of the research facility and were provided with ad-libitum access to growers’ mash of vital® feed and were also supplied with water.

Chemicals and Reagents

High-performance liquid chromatography (HPLC)-grade solvents, including acetonitrile, Milli-Q water, and glacial acetic acid, were employed in the experiment. The polyphenol standards utilized in this study were procured from Sigma Chemical Co. (St. Louis, USA) and possessed purities exceeding 95%. The chemicals utilized in this investigation were of analytical grade and were obtained from Sigma Aldrich, a company based in St. Louis, MO, USA.

Plant Collection and Extraction

Fruits of Tamarindus indica (tamarind) were bought in November 2019 from Potiskum Central Market, Potiskum Local Government Area of Yobe State, Nigeria. The fruits underwent authentication at the Department of Botany, Faculty of Science, University of Maiduguri. The tamarind fruits were subjected to a drying process in a shaded environment. Subsequently immersed in warm water for a duration of 15 to 20 minutes with gentle smashing. After that, it was subjected to squeezing to extract its contents. Subsequently, the extract was strained through a sieve, with a receptacle positioned underneath to collect the pulp. A spoon was employed to agitate and provide pressure, facilitating the extraction of a maximal amount of tamarind pulp or paste. The seeds, membrane, and fibers were discarded. The tamarind extract was transferred into a glass beaker, which was subsequently sealed with foil paper and placed in a fridge for storage at 4ºC for future utilization.

HPLC Analysis

The investigation involved the utilization of high-performance liquid chromatography (HPLC) to analyze the aqueous of tamarind fruit pulp. The chromatographic system employed for this study was the YL 9100, manufactured in Korea, which comprised an autosampler (YL 9150) equipped with a fixed loop of 100µl and a YL9120 UV-visible detector. The separation procedure was conducted using a ProteCol column (C18G, 250mm x 4.6 mm, 5µm) at room temperature. The mobile phase utilized in this study comprised of acetic acid acidified deionized water with a pH of 2.8 as solvent A and acetonitrile as solvent B. The separations were carried out utilizing the isocratic mode, with elution done at a flow rate of 1 ml/min. The samples underwent a 15-minute run time, and detection was performed using a UV detector at a wavelength of 254nm. The chromatographic results were obtained and analyzed using the autochrome-3000 program.

Experimental Design

A cohort of 42 apparently healthy albino rats, with weights ranging from 150g to 200g, was randomly allocated into six groups, each consisting of seven animals.

Grouping of Experimental Animal

Group 1: Traumatized and treated with 100mg/kg tamarind extract.
Group 2: Traumatized and treated with 200mg/kg tamarind extract.
Group 3: Traumatized and treated with 400mg/kg tamarind extract.
Group 4: Traumatized and treated with 800mg/kg tamarind extract.
Group 5: Traumatized, non-treated.
**Induction of Traumatic Brain Injury**

The experimental animals, except for those in the negative control group, were subjected to head injury using the weight drop method. This approach involved the use of an acceleration impact device developed by Marmarou et al. (1994). The experimental rats were appropriately immobilized and anaesthetized using Xylazine and Ketamine combination at a dosage of 5mg/kg and 80mg/kg body weight, respectively. The animals were classified using the following designations: Neck, Back, RFL, RHL, Tail, LFL, and LHL. After the rats have lost consciousness, their scalp was shaved using a razor blade and subsequently sterilized with an antiseptic solution (Dettol). The cranium was surgically exposed by a midline incision measuring 1cm, utilizing a scalpel blade and a blade holder. Subsequently, a stainless-steel disk with dimensions of 10mm in diameter and 3mm in depth was affixed to the center of the frontal bone. The experimental animals were immobilized in a supine position on a foam bed with a depth of 10cm. The injury was caused by releasing a brass ball, weighing (120g) through a guide from a vertical distance of one meter. The stainless-steel disc was promptly removed from the cranium, while the lacerated skin was sutured utilizing chromic catgut size 3.0 in a simple interrupted suture pattern. The animal was permitted to recuperate within the confines of the enclosure.

**Reconstitution of the Extract and treatment**

One gram (1g) of tamarind extract was dissolved in 20 ml of distilled water (pH 7.5) to give a concentration of 50mg/ml and administered to groups I & II rats through the oral route. One gram (1g) of tamarind extract was dissolved in 5ml distilled water to form a concentration of 200mg/ml and administered to groups III & IV rats orally. Each TBI-induced rat was treated using a standard formula for calculating the volume of the drug, as highlighted below. The treatment was done once daily for three weeks after TBI.

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Volume\ (mls) = \frac{Bodyweight \times Dosage}{Concentration}
\]

**Neurological Severity Score for Rats**

The scoring was done daily for 3 weeks using the methods as described by Yarnell et al. (2016). Two vacant containers were positioned with approximately 25cm between them, a linear balance beam was positioned on top of the containers. Subsequently, a rat was positioned at the initial point of the beam to carry out a series of neurobehavioral assessments while accounting for the element of time. These assessments encompassed various tests, namely the general balance test, landing test, sound reflex, tail raise test, drag test, righting reflex, ear reflex, eye reflex, and paw flexion reflex. The findings are documented in the following manner.

- 0 = rat was able to do each of the above tests successfully without any hindrance.
- 1 = The rat had some difficulties while undergoing those tests.
- 2 = rats did not respond. The scale has a total of 0 to 20 scores with higher scores indicating an increase in severity.

**Memory Function Test**

Novel object discrimination test was used to assess the memory function as reported by (Yarnell et al. 2016) A box with bedding was placed on a flat surface. Inside the box there are objects of the same colour, size, and shape. Object 1 was identified as the familiar object, and object 2 was identified as the new object. Object 1 was placed first, and the rat was introduced. The rat was allowed to familiarize itself with the object for 5 minutes and then removed. Object 2 was then placed 5cm away from object 1. The rat was also observed for another 5 minutes. The rat was seen playing with objects, and two stopwatches were used to record the time spent with object 1 and object 2 simultaneously. The amount of time spent on each object was recorded on the score sheet.

**Brain Extraction**

The rats were sacrificed under anesthesia and the brain was extracted in accordance with the methodology outlined by Keshteli et al. (2014), employing micro dissecting techniques. A midline incision was performed on the head, separating the skin. The incision was made from the roof of the skull to the mid-eye area, ensuring that the cut did not penetrate the brain tissue, and the skull was divided along the midline fissure. The cranial cap was removed using curved forceps, applying a delicate amount of pressure. The third step in the process entailed the removal of the brain from the cranial cavity with a surgical approach. The micro spatula is a laboratory tool commonly used for precise and delicate handling of small amounts of substances) situated inferiorly and extending over the entirety of the cranial cavity, encompassing the olfactory lobes to the proximal segment of the spinal cord. After carefully transferring the brain to a Petri plate with a diameter of 60 mm, the tissues underwent a rinsing process using phosphate-buffered saline (PBS) to eliminate any presence of red blood cells and clots.

**Histopathological Examination**

The brain tissue samples obtained from all the experimental animals were immersed in a 10% buffered formalin solution for a duration of 48 hours. The fixed tissues underwent dehydration in alcohol samples, which were subsequently categorized according to their concentration levels, namely 70%, 80%, 90%, and 100%. Utilizing an automated tissue processor. The tissue samples were subjected to a clearing process utilizing xylene-embedded tissues and a microtome knife affixed to a microtome apparatus. Subsequently, the sliced tissue sections were carefully placed onto a grease-free and pristine surface for further analysis. The glass slide was exposed to the surrounding environmental conditions to facilitate the drying process, and later underwent a staining procedure with hematoxylin and eosin stain (H and E). The histological slides were examined on microscope under varying magnifications (x4, x 10, x20 and x40) (Maynard and El-Nageh, 2003).

**Statistical Analysis**

The data obtained were presented in the format of mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used with turkey posthoc to detect significant differences between groups. Significance was attributed to differences in p values that were less than or equal to 0.05 (P ≤ 0.05).
Ethical Statement
The Animal Use and Ethic Committee of the Faculty of Veterinary Medicine University of Maiduguri has approved the work, with AUP number AUP-R001/2022

RESULTS
Phenolic and Flavonoid Content of Tamarind Fruit Pulp Extract
The presence of rutin flavonoids, caffeic acids, and tannic acids in the tamarind fruit pulp extract is shown by the identification of distinctive peaks. These peaks were determined by comparing their retention durations (4.98, 3.6, and 2.63) with those of legitimate standards. This is demonstrated by the chromatogram presented in Figure 1.

Neurological Severity Score (NSS) of Albino Rat Induced with TBI
The results of the Neurological Assessment for each experimental group are displayed in Figure 2. There were significant improvements in the neurological response in the rats treated with the tamarind fruit pulp extract, as indicated by their NSS from weeks 1 to 3. As a result, the finding demonstrates a significantly effective level of reduction in the severity of TBI in rats.

Memory function test in TBI rats treated with Tamarind fruit pulp.
The results of the novel object discriminating index are shown in Figure 3. The results demonstrate that in week 1, rats spent more time with the familiar object (FO) than the new object (NO), which tends to yield results towards the negative axis, whereas, in week 2, rats spent more time with the new object (NO) than the familiar item (FO).

Histopathological results
The most obvious gross lesions were observed in the whole of Group 5 and a few of Group 4. Histological changes of albino rats subjected to traumatic brain injury treated with tamarind fruit pulp extract, including Group 1, show oligodendroglia exhibiting increased perinuclear halo and a few dead neurons (Figure 4). Groups 2 and 3 presented a few spongiosis and few dead neurons with moderate perineuronal vacuolation (Figure 5). Group 4 shows red shrunken neurons (Figure 7) and diffuse spongiosis (Figure 5). Group 5 presented diffused areas of central chromatolysis (Figure 8), while the normal group 6 showed normal neurons and various glial cells (Figure 9).

DISCUSSION
Plant-active compounds are gaining popularity due to their bioactive properties and have the potential to be used as alternative sources of treatment. The tamarind fruit pulp extract used in this study contains alkaloids 17.72 mg/g, flavonoids 17.48 mg/g and tannin 10.35 mg/100g in the HPLC analysis (Figure 1). This can be the major reason for its significant amount of antioxidant activity reported. Plant phenolic components are a significant source of phytochemicals that promote health and are a vital class of biologically active substances that support health and fight
disease (El Gharras, 2009). Very potent antioxidant effects are produced by phenolic substances (Scalbert et al., 2005). Numerous epidemiological studies have provided compelling evidence of the significant association between the intake of phenolic-rich foods and a decreased likelihood of developing cardiovascular and neurological illnesses (Weichselbaum et al., 2010). Furthermore, recent research suggests that the flavonoid component of tamarind fruit pulp may have particularly potent effects on rats' cognition and reverse memory deterioration (Spencer, 2010).

Figure 5: Photomicrograph of section of a brain (cerebrum) of a rat in group 2 TBI-induced treated with 200 mg/kg body weight tamarind for 3 weeks, showing few spongiosis (arrow heads), H & E, X400

Figure 6: Photomicrograph of section of a brain (cerebrum) of a rat in group 3 TBI-induced treated with 400 mg/kg body weight tamarind for 3 weeks, showing few dead neurons with perineuronal vacuolations (arrows), H & E, X400

Figure 7: Photomicrograph of section of a brain (cerebrum) of a rat in group 4 TBI-induced treated with 800 mg/kg body weight tamarind for 3 weeks, showing red shrunken neurons (arrows), H & E, X400

Figure 8: Photomicrograph of section of a brain (cerebrum) of a rat in group 5 TBI-induced nontreated showing diffused areas of central chromatolysis (arrows), H & E, X400

Figure 9: Photomicrograph of section of a brain (cerebrum) of a rat in group 6 (normal group) showing normal neurons (arrows) with various glial cells (arrow heads), H & E, X400.

This study demonstrated that tamarind fruit pulp extract has a neuro-therapeutic effect on albino rats induced with traumatic brain injury (TBI), as the rats showed an improved neurological function from the first week to the second and third week against the severity of the injury as shown in Figure 1. The observed restoration of neurological functions in the groups treated with tamarind fruit pulp extract may be attributed to its potential to mitigate oxidative damage. This is significant as traumatic brain injury (TBI) leads to structural and functional impairments due to the presence of oxidative stress, which subsequently impacts neurological function. (Davis, 2000). According to Grimm et al. (2016), oxidative stress results in a general deterioration of cellular functions. There have been reports of ROS-related damage in the specific area of the brain that is experiencing neurodegeneration (Ikeda et al., 2002). Tamarind fruit pulp used in this study, contains antioxidant molecules (phenolic acids) possessing the capacity to engage in interactions with free radicals, interrupting the chain reaction before the infliction of harm onto essential molecules. (Siddhuraj and Backer, 2007). It has been reported that flavonoids, one of the constituents of tamarind fruit pulp, can safeguard neurons that are susceptible to damage and augment the performance of pre-existing neural structures (Jeremy, 2010; Kushner, 1998). Hence, it is plausible that the enhanced neurological function observed in the group receiving treatment for trauma may be attributed to the antioxidant properties of the extract derived from tamarind.
fruit pulp. According to Khanzada et al. (2008), tamarind fruit pulp possesses vitamins C and E, leading to enhancements in brain function and the provision of neuroprotection. The findings of this study concur with those of Zemlan et al. (1989).

TBI induction caused memory impairment in the rats. This is demonstrated by the lower discrimination index (DI) of the traumatized rats in the first week compared to the second week after treatment. One of the reasons could be a decrease in CBF, which causes oxidative stress and cell death due to inadequate perfusion (Noppen et al., 2004). The outcome of Figure 2 demonstrates that albino rats given varied doses of tamarind fruit pulp extracts showed an improvement in their memory function while that of the traumatized non-treated rats (TNT) did not have significant improvement. This might be a result of tamarind's antioxidant action against ROS. According to Vyas et al. (2009), tamarind fruit pulp is very effective in trapping free radicals and preventing them from forming lipid hydro-peroxide that can be produced during TBI. As described in numerous animal studies, the different antioxidants included in tamarind fruit pulp work directly on ROS and are promising in modulating oxidative stress (OS) pathways and boosting memory outcomes (Zemlan et al., 1989; McCall and Frei, 1999).

The observed intracranial haemorrhage (contusion) in this study is classified as focal (Mckee and Daneshvar, 2015) and indicates severity of the injury, which may cause death in most cases (Finnie, 2016; Rahaman and Delbigio, 2018). The age of brain contusions can be determined with reasonable accuracy from routinely stained histological sections (Loberg and Torvik, 1989) The traumatized non treated (TNT) group, showed neurodegeneration and necrosis characterized by dead neurons, diffuse spongiosis, red shrunken (eosinophilic) neurons and central chromatolysis in the cerebrum, indicating that the induction caused diffuse brain injury as described by Andre et al., (2014). Many of these lesions have been used to determine the onset of injury. Eosinophilic neurons have been observed in many surviving cases in less than an hour after injury (Anderson et al., 1998). Similarly, shrinkage and pyknosis of dying neuron have been reported to be apparent as early as 30 minutes post-injury and persist for up to one day (Rahaman and Delbigio, 2018). The shrunken neurons reported in this study were observed three weeks after induction of injury, meaning that neurodegeneration has been on going, most likely because of persistent generation of free radicals. These lesions were, however, not pronounced in the group treated with tamarind fruit pulp extract which could be due to the restorative or normalization process of the extract. These findings provide empirical justification for a long-standing use of the tamarind fruit pulp. However, more investigation is required to elucidate the precise mechanism(s) of action and isolate the bioactive constituents responsible for the Neuro-therapeutic effect of the extract.

**Conclusion**

The weight drop approach of causing traumatic injury, according to the study, results in neurological deficits and histological changes. Administration of tamarind fruit pulp extract, however, improved neurological function and reduced neuropathology while improving functional recovery in rats after TBI. The increased neurological and memory functions of the traumatized and treated rats may have contributed to the neuro-therapeutic benefits of the tamarind fruit pulp extract. In contrast, the TNT animals did not exhibit any improvement in these functions. These findings imply that supplementing antioxidants may be a helpful neuro-therapeutic approach to treating secondary injuries brought on by TBI.

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**Conflict of Interest**

The authors have no conflict of interest to declare.

**Author Contributions**

IB and NS conceptualized and designed the study, PAM and BU collected samples, analyzed data and drafted the manuscript of this research; MBBM provided critical advice on study design, data acquisition and skills on the laboratory activities; MUS, OOU and AH carried out the acquisition of retrieved data, final review and editing of the manuscript; AW, KAS, and MBBM supervised the study, interpreted data, analyzed data and reviewed the document. All authors have reviewed and given their approval of the final version of the work.

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