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Raffia hookeri Ethanolic Pulp Extract Ameliorated Neuronal Damage and Brain Oxidative Stress Following Mechanical-Induced Traumatic Brain Injury in Rats

Owoeye O.[1], Awoyemi F.O.[1], Imosemi I.O.[1], Atiba F.A.[1] and Malomo A.O.*[1,2]
Departments of [1]Anatomy and [2]Surgery, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: Traumatic brain injury (TBI) is a complex process resulting into structural brain damage and functional deficits as a result of an external mechanical force. This study aimed to investigate the possible ameliorative effect of *Raphia hookeri* ethanol extract (RHEE) on induced acute traumatic brain injury in rats. The choice of the plant was based on its reported anti-oxidative property. Thirty-six female Wistar rats were divided into six groups of six animals each. I: CONTROL - distilled water orally; II: RHEE - 100 mg/kg RHEE; III: Sharp trauma brain injury (STBI); IV: STBI+RHEE; V: Blunt trauma brain injury (BTBI); VI: BTBI+RHEE. Brain injury was inflicted using modified weight drop technique on experimental day 1 while RHEE was given orally by gavage for 7 days post-injury. Blood was collected serially 24hrs, 72hrs and 7 days post-trauma for full blood count and differentials of the white blood cells. On day nine, rats were euthanized and brain harvested for biochemical and histological analyses. Trauma significantly ($p<0.05$) reduced the relative brain weight of rats compared with the control. Lymphocyte count increased while neutrophils reduced in all traumatized rats compared with control group. Both BTBI and STBI significantly ($p<0.05$) elevated MDA and significantly ($p<0.05$) reduced the level of GSH, the activities of SOD and CAT enzymes compared with control group. Histologically, the extent of haemorrhage into the subarachnoid and brain parenchyma in STBI and BTBI groups was reduced in the BTBI+RHEE and STBI+RHEE groups. Administration of RHEE reduced oxidative damage and ameliorated neuronal damage in sharp and blunt brain injuries.

Keywords*:* Raphia palm fruit, induced brain injury, oxidative stress, white blood cells, cerebral cortex, haemorrhage.

**Address for correspondence*: ademalomo@yahoo.com; oowoeye@gmail.com. Phone: +2348034852526.

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INTRODUCTION

Traumatic brain injury (TBI) is damage to the brain resulting from an external mechanical force leading to temporary or permanent impairment of cognitive, physical and psychosocial functions (Maas et al., 2008; Gaetz, 2004). Traumatic brain injury can be classified as primary which occurs immediately after trauma, and secondary which may appear several hours or even days later (Ozdemir et al., 2005). The structural damage and functional deficits following TBI are due to both primary and secondary injury mechanisms (Davis, 2000). The primary injury is characterized by the immediate mechanical disruption of brain tissue at the time of exposure to the external force and the release of the chemical mediators which act on the neighbouring cells thus promoting further cell loss (Yamaura et al., 2002; Park et al., 2004). This further cell loss and other pathological mechanisms of metabolic, cellular and molecular events such as excitotoxicity, ionic imbalances, inflammatory response and oxidative stress which evolve over minutes to months after the primary injury is referred to as the secondary injury (Lenzlinger et al., 2001; Arundine and Tymianski, 2004; Marklund et al., 2006; Bramlett and Dietrich, 2007; Guimaraes et al., 2009). Although the interplay of three major deleterious pathways namely: glutamate excitotoxicity, Ca^{2+} overload, and oxidative stress is believed to be responsible for the damage and neuronal death following TBI (Algattas and Huang, 2014), we examined the effects of only the latter in this study. The effective prevention of the variety of pathophysiological processes such as anti-inflammatory and anti-oxidative strategies is one of the key factors for improving the prognosis of TBI patients (Stelmasiak et al., 2000; Zhen-Guo et al., 2013). The devastating consequences of TBI and its pathophysiology require an effective treatment and this requires the exploration of plant products with antioxidant capability since plants are easily accessible, less toxic and less expensive and one of such is *Raphia hookeri*.

Raphia hookeri is a member of Palmaceae family that grows in the eastern and western parts of Nigeria. Its fruit is cone-shaped with an outer layer of rhomboid-triangular and overlapping reddish brown scales, a middle yellow, mealy, oil-bearing mesocarp and inner single hard nut (Mbaka et al., 2013). It has

been demonstrated to have medicinal and therapeutic properties warranting its use in herbal medicine in the treatment of various illnesses (Ogbuagu, 2008). Its seed reportedly attenuated hyperglycaemia and ameliorated dyslipidaemia (Mbaka et al., 2012) and has also been shown to effectively attenuate hyperplasia and reduced the size of enlarged prostate gland that was exogenously induced *via* its anti-oxidative activity (Mbaka et al., 2013). An earlier investigation showed that the pulp contains high concentrations of vitamins A and E, niacin, alkaloid, saponins, flavonoids and phenols all of which might enhance its antioxidant potentials (Edem et al., 1984).

The effect of brain injury will depend on the part of brain that is injured since different parts of the brain controls different functions although there is an overall synergy of the activities. In this study, we injured the frontal motor cortex of rats and thereafter observing the effect of *Raphia hookeri* ethanolic extract (RHEE) on the consequences of this induced injury in rat brain biochemically and histologically as well as on the blood profile.

This study is aimed at investigating the potential ameliorative effects of RHEE on the extent of induced acute traumatic brain injury in the frontal motor cortex of female Wistar rats and thus answer the research question of whether RHEE can protect rat brain from induced TBI.

MATERIALS AND METHODS

Plant extract processing

Raphia hookeri fruit was obtained from the swamps of Oke Odan, Apete, Ibadan, Oyo State and authenticated at the Forestry Research Institute of Nigeria (FRIN), Jericho, Oyo State, Nigeria with reference number FHI/110540. The hard, tough and scaly exocarp of the fruits were removed and discarded and the soft, mealy mesocarp (pulp) scraped from the seeds. The pulp of *R. hookeri* fruits was air-dried and grinded into powdery form for phytochemical screening and extraction at the Department of Pharmaceutical Chemistry, University of Ibadan, Nigeria. About 980 g of the grounded plant material was transferred into a glass container and 7 litres of absolute ethanol added, stirred at 2 hours intervals for 5 minutes hours and allowed to stand for 72hrs. The mixture was filtered in muslin bag followed by Whatman filter paper and the filtrate was concentrated using rotary evaporator set at 40^{o}C. The final aqueous extract of 79.24 g gave a percentage yield of 8.1% and was termed *Raffia hookeri* ethanolic extract (RHEE).

Animals and animal ethics

Thirty-six adult female Wistar rats weighing between 150-200 g were obtained from the College of Medicine Animal House, University of Ibadan and were randomly assigned into control and experimental groups. Thereafter, the rats were allowed 9 days to acclimatize to the naturally illuminated animal house of the Department of Physiology, University of Ibadan, with access to feed and water *ad libitum*. The animals were housed in transparent plastic cages with wood shavings as bedding. All of the animals received humane care according to the conditions stated in the 'Ethics Guiding the Care and Use of Laboratory Animals' published by the US Department of Health and Human Services, Washington (PHS, 1996).

Experimental design and animal treatment

The rats were randomized into six groups of six animals each as follows:

I: CONTROL administered distilled water.
II: Administered 100 mg/kg RHEE.
III: Sharp traumatic brain injury (STBI) induced.
IV: STBI induced plus 100 mg/ kg RHEE.
V: Blunt traumatic brain injury (BTBI) induced.
VI: BTBI induced plus 100mg/kg RHEE

Trauma was inflicted on day 1 of experiment and RHEE administered daily for 7 days by oral gavage according to the method of Mbaka *et al. (*2012).

Induction of Traumatic Brain Injury

After anaesthesia using 100 mg/kg body weight of Ketamine chloride injection, the hair on the animal's head was shaved off to expose the target area and methylated spirit used to clean the shaved area for antisepsis. Animals were then placed on the levered table of the modified weight drop device shown in Figure 1. A spot of injury was located, 2.5 mm posterior and 2.5 mm lateral to the bregma (Feeney et al., 1981). A round metallic ball fashioned to the end of Steinmann's pin was used for blunt type of TBI. Another Steinmann pin was fashioned to induce sharp type of TBI. The levered table was adjusted to accommodate 2.5 mm traumatic distance for each animal. A metallic object of 425 g was dropped at a uniform height of 5 cm on the skull to induce both blunt and sharp brain injury. Topical application of cotton wool soaked with methylated spirit on the bleeding spot prevented skin contamination. Finally, each animal was removed from the levered table and gently placed in the cage to recover.

TBI Inducing Apparatus (Modified Weight – Drop Device)

The weight-drop technique of Farran et al., (2014) was modified. Briefly, a retort stand held two clamps at a distance of 5cm interval, the upper clamp held a wooden block called "stopper" used to regulate the traumatic distance by stopping the Steinmann's pin (Figure 1). The lower clamp held a wooden block called "Guide" which was used to guide the Steinman's pin pathway over the rat's skull (this replaced the "Guide tube" of Farran et al.,2014). A levered table was made from a modified car jack with

a flat board on it on which the sedated animal was laid in a prone position to replace the plastic case used by Farran et al., (2014).

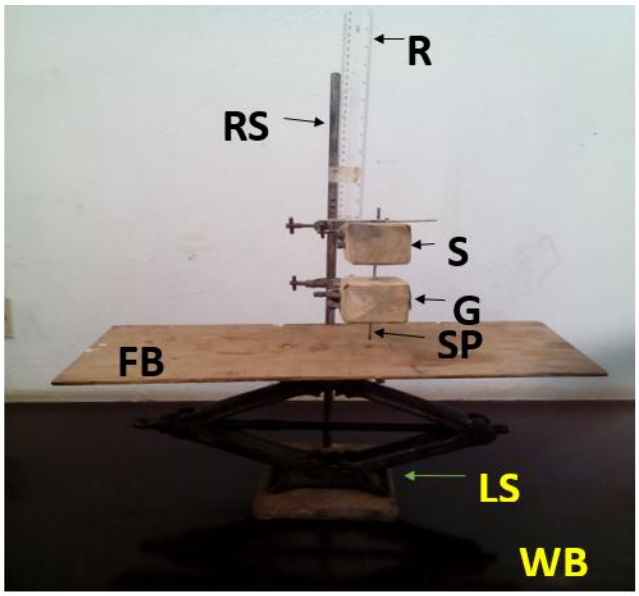

Fig. 1: Picture of the "weight drop" apparatus modified after Farran et al., (2014). R - Ruler, RS - Retort Stand, LS - Lever Stand, WB - Work Bench, FB - Flat Board, SP - Steinmann's Pin, G - Guide, S – Stopper.

Haematological Test

Blood samples were collected from the periorbital space of each rat into the heparinized bottle for estimating total white blood cell count, and the differential cell count on days 1, day 3 and day 7 post-traumatic injury. The blood was analysed using an Auto-haematological Analyser Machine at the Haematology Laboratory of the Department of Veterinary Medicine, University of Ibadan.

Animal sacrifice and biochemical assays

On day nine of the experiment, rats were euthanized with Ketamine overdose. Brain samples were collected, rinsed, weighed and divided into two halves. One half of the brain was preserved in 10% formalin for histological study while the other half of the brain for biochemical study was preserved in phosphate buffered solution PBS at pH 7.4 according to the method of Owoeye and Salami (2015). This part was were homogenized and homogenates centrifuged with cold centrifuge (4 °C at 12,000 rpm for 10 – 15 minutes). The supernatant was collected for the estimation of Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT). The level of MDA determined lipid peroxidation according to the method described by Varshney and Kale (1990), whereas GSH was determined at 412 nm in a colorimeter using the method described by Beutler *et al.*, (1963). The SOD activity was determined by the method described by Del-Maestro et al. (1983), while CAT activity was measured spectrophotometrically at 570 nm by the method of Sinha (1972). The fixed brain tissues were processed at the Histology Laboratory, Department of Anatomy University of Ibadan, Nigeria. Rats' brain specimens were processed through the stages of fixation, dehydration, clearing, infiltration, embedding and thereafter sectioned at 6 µm thickness with a Rotary Microtome (Leica RM2125 RTS, Germany). The ribbons were stained with haematoxylin and eosin and cresyl violet according to the method of Bancroft and Gamble (2008) to demonstrate general histology of the brain and possible microscopic alterations.

Statistical analysis

All the data were expressed as the mean ± Standard Deviation. Data was analyzed using Student's t-test and one-way ANOVA using Graph pad Prism (Version 7.00). Confidence interval was calculated at 95% and level of significance set at 5%.

RESULTS

Phytochemistry

The phytochemical screening of RHEE showed the presence of alkaloids, flavonoids, terpenoids, saponins, anthraquinones and tannins, while cardiac glycosides was absent.

General observations

***Body and brain weight changes*:** As shown in Table 1, brain trauma caused a significant ($p<0.05$) reduction of percentage weight differences in all the groups compared with the control. Similarly, the relative brain weight was significantly lower ($p<0.05$) in all the traumatized rats when compared with the control with the exception of BTBI group (Fig. 2).

Table 1: Body weight changes of the animals

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)	% Weight difference
CONTROL	168.5±8.01	184.0±9.01	15.5±0.50	9±0.15
RHEE	167.0±5.87	169.0±6.63	2.0±0.01	1±0.01*
STBI	224.7±15.06	227.2±15.57	2±0.01	1±0.01*
STBI+RHEE	229.2±16.46	223.5±12.20	-5.7±0.21	-2±0.01*$^{\#}$
BTBI	185.2±8.51	192.2±13.55	7±0.23	4±0.02*
BTBI+RHEE	249.7±18.48	244.5±18.96	-5.2±0.21	-2±0.01$^{\#}$

RHEE, *Raffia hookeri* ethanolic extract; STBI, Sharp Traumatic Brain injury; STBI+RHEE, Sharp Traumatic Brain Injury +RHEE; BTBI, Blunt Traumatic Brain Injury; BTBI+RHEE, Blunt Traumatic Brain Injury±RHEE. Data are expressed as mean ± S.D. for 6 rats per group. *P<0.05 versus CONTROL, *$^{\#}$P< 0.05 versus STBI, $^{\#}$ P< 0.05 versus BTBI.

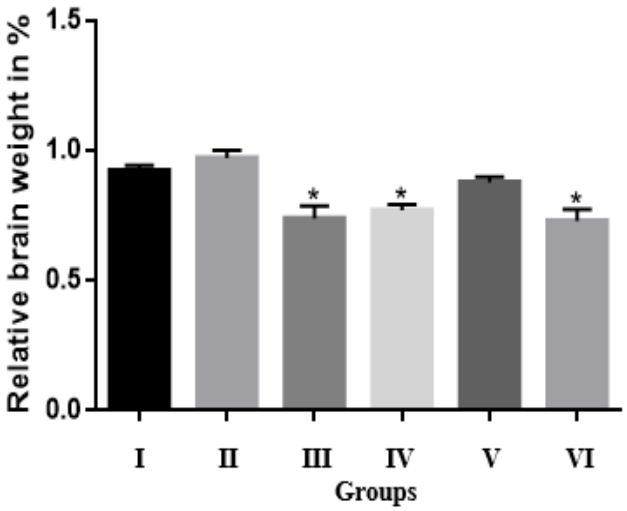

Figure 2: Relative weight of the brain of rats in I= Control; II=RHEE; III=STBI; IV=STBI+RHEE; V= BTBI; VI= BTBI+RHEE. *P<0.05 versus CONTROL.

Table 2(A): Effect of treatments on the total white blood cells of rats on Days 1, 3 and 7 post-trauma

Groups	Total White Blood Cell count (10^3/µL)		
	Day 1	Day 3	Day 7
I	4575±1312	4565±1302	4573±1222
II	3775±528	3765±524	3774±523
III	3525±493.7	6883±2289*	4508±565.2
IV	2967±375.1*	6075±1778#*	4892±572.2#*
V	4100±1305	5392±951.5#*	3608±385.2#*
VI	3117±531.7*	5875±1188#*	4708±900.8#*

I= Control; II=RHEE; III=STBI; IV=STBI+RHEE; V= BTBI; VI= BTBI+RHEE. *P<0.05 versus CONTROL; # P< 0.05 versus BTBI or STBI, *# P<0.05 versus value for previous day.

Table 2(B): Effect of treatments on the Neutrophil of rats on Days 1, 3 and 7 post-trauma.

Groups	Neutrophil Count (%)		
	Day 1	Day 3	Day 7
I	41.17±14.5	41.83±17.6	39.33±16.2
II	30±12.3	31.83±4.6	31.83±4.6
III	29.67±4.6*	27.5±5.4*	32.83±5.9
IV	26.17±2.8*	28.83±5.1*	29.67±5.1*
V	25.33±4.1*	31.83±5.0*	29.83±10.1*
VI	28.83±5.5*	27.33±4.8*	31.33±8.9*

I= Control; II=RHEE; III=STBI; IV=STBI+RHEE; V= BTBI; VI= BTBI+RHEE*P<0.05 versus CONTROL.

Table 2(C): Effect of treatments on the Lymphocytes of rats on Days 1, 3 and 7 post-trauma.

Groups	Lymphocytes counts (%)		
	Day 1	Day 3	Day 7
I	62±4.7	61.67±10.4	63.5±7.0
II	67.83±12.8	64.5±4.2	64.5±4.2
III	66.83±4.4	68.5±6.0	63.5±5.2
IV	70±2.6*	67±4.6	67.17±6.3
V	70±4.7*	63.5±4.6	67.17±11.5
VI	67.5±5.6	69.5±4.4	65.5±10.4

I= Control; II=RHEE; III=STBI; IV=STBI+RHEE; V= BTBI; VI= BTBI+RHEE. *P<0.05 versus CONTROL.

Table 3 **The effects of RHEE on the antioxidant defense mechanisms in brain of rats**

Groups	MDA (µmoles/mg protein)	SOD (µUnit/mol)	GSH (µg/ml)	CAT (µmol/min^{-1} mg^{-1})
I	1.71±0.1	3.73±0.6	59.50±4.7	6.22±0.8
II	1.91±0.1	2.73±0.4	60.01±5.2	5.01±0.7
III	6.22±0.8*	1.51±0.1*	28.41±3.3*	1.81±0.0*
IV	2.53±0.4#	0.81±0.0#	37.31±2.9*	4.13±0.9#
V	4.31±0.8*	2.2±0.1*	39.20±2.9*	1.92±0.2*
VI	1.87±0.1##	2.0±0.1*	48.41±3.2*	2.92±0.3*

I=Control; II=RHEE; III=STBI; IV=STBI+RHEE; V= BTBI; VI= BTBI+RHEE. Data are expressed as mean ± S.D. for 6 rats per group. *P<0.05 versus CONTROL, #P< 0.05 versus STBI, ## P< 0.05 versus BTBI.

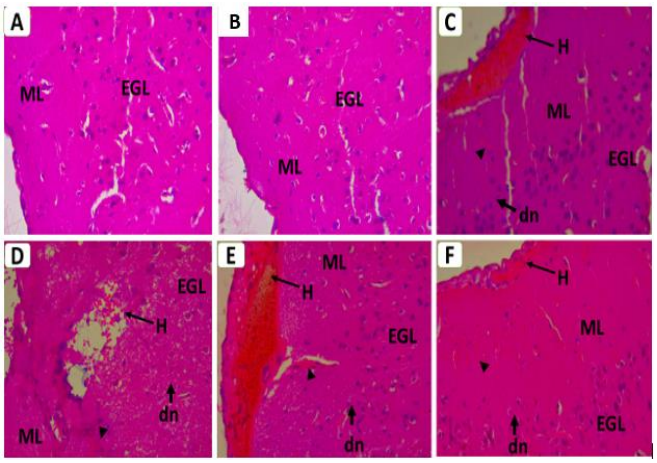

Figure 3: Representative stained sections of the cerebral cortex of rats in CONTROL(A), RHEE (B), STBI (C), STBI+RHEE (D), BTBI (E) and, BTBI+RHEE (F). Portions of the molecular and external granular layers of the cortex show evidences of haemorrhage (H). Blood extravasation into the parenchyma of the cortex is depicted with arrowheads. Normal neurons indicated as "n", while degenerated cortical neurons (dn) are deep to areas of haemorrhage. ML, Molecular layer; EGL, External Granular Layer**.** H&E stain, x400.

The effects of RHEE on the antioxidant defense mechanisms in brain of rats

Table 3 shows the effects of RHEE on the antioxidant defense system and biomarkers of oxidative stress in brain of trauma-treated rats. BTBI caused a significant (0.05) elevation in the level of malondialdehyde (MDA), and reduction in glutathione (GSH) level. However, it caused a reduction the level of glutathione and activities of SOD and CAT when compared with the control. The co-administration of RHEE ameliorated the perturbations in these parameters by restoring them to near control levels as shown in the table.

Histological evaluation of normal and traumatized cerebral cortex of rats.

Figure 3 depicts our findings of the traumatized and non-traumatized cerebral cortices of the experimental rats. Portions of the molecular (ML) and external granular layers (EGL) of the cortex show evidence of haemorrhage (H) as shown in Figures 3C, 3D, 3E, and 3F. Blood extravasation into the parenchyma of the

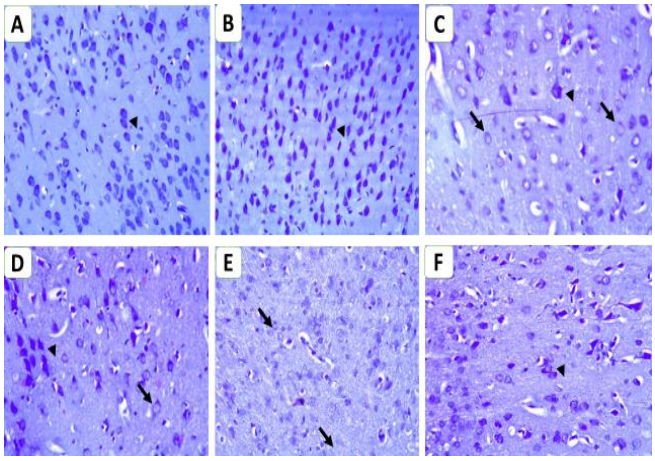

Figure 4: Representative stained sections of the cerebral cortex of rats in CONTROL(A), RHEE (B), STBI (C), STBI+RHEE (D), BTBI (E) and, BTBI+RHEE (F). Portions of the external granular layers of the cortex deep to injured part to show neuronal response to injury. Normal staining of cortical neurons in groups (arrowheads) while pale staining neurons (arrows) are prominent in STBI and BTBI groups. ML, Molecular layer; EGL, External Granular Layer**.** Cresyl violet stain, x400.

cortex is denoted with arrowheads. Normal neurons indicated as "n", while degenerated cortical neurons (dn) are deep to areas of haemorrhage. The cresyl violet stain (Figure 4) demonstrated that degenerated cortical neurons deep to the injured part of the brain were more pronounced as pale staining cells in the STBI and BTBI compared with STBI+RHEE and BTBI+RHEE groups respectively.

DISCUSSION

The present investigation provided biochemical, haematological and histological data which suggested that post-traumatic treatment of rats with *Raffia hookeri* ethanolic extract (RHEE) mitigated the adverse effects of sharp and blunt trauma in experimental rats. The RHEE prevented trauma-induced oxidative stress and cerebral cortical neuronal death, additionally, it augmented brain antioxidant defense mechanisms and ameliorated neuron damage in traumatized rats.

The reduction in the body weight of rats co-treated with RHEE after trauma (STBI+RHEE and BTBI+RHEE) might be due to the effect of the trauma resulting in reduction in activity and poor feeding due to loss of appetite. The reduction in the relative brain weight in all traumatized rats might also be secondary to the trauma.

The haematological analysis was used to measure inflammatory cell response in TBI. It has been reported that CNS expression of pro-inflammatory cytokines and complement components leads to recruitment of peripheral inflammatory cells (neutrophils and monocytes ⁄macrophages) across the blood brain barrier and enhancement of the established neuro-inflammation (Scholz et al., 2007; Szmydynger-Chodobska et al., 2012). Studies showed that after focal TBI, peripheral inflammatory cells increase in the CNS, neutrophils in particular arrive within one hour of TBI and reaches its peak by third day, and then disappears or decreases rapidly with time (Kriz, 2006; Yilmaz and Granger, 2008; de Rivero Vaccari et al., 2009). Although the total white cell count peaked on day3, the neutrophils count was not elevated whereas the lymphocyte count was raised contrary to the report of Weckbach at al. (2012) who reported that lymphocytes did not play a major role in TBI pathogenesis.

The presence of flavonoids and tannins in RHEE suggest that it might have antioxidant potential (Ayoola et al., 2008), since they are phenolic compounds which act as primary antioxidants or free radical scavengers (Dada et al., 2017). The elevated level of MDA and reduction of GSH level indicated oxidative stress in both STBI and BTBI rats which was supported by previous findings that oxidative stress occurs in traumatic brain injury (TBI) ((Inci *et al.*, 1998; Webster et al., 2015). Oxidative damage has been associated as one of the principal factors accompanying the secondary injury mechanisms and changes that worsens the outlook of TBI (Ozdemir et al., 2005). Brain tissue is known to be highly sensitive to damage by free radicals because of its high concentration of polyunsaturated fatty acids, low concentration of cytosolic antioxidants and high use of oxygen (Ebokaiwe *et al.*, 2013).

Animals in groups post-treated with RHEE had their MDA levels reduced while the level of GSH was elevated suggesting antioxidant activity of RHEE to neutralize or mitigate the oxidative damage of both sharp and blunt trauma on the rat brain. Similarly, the activities of SOD and CAT that were reduced by TBI was elevated by post-trauma treatment with RHEE. The results suggested that RHEE demonstrated ameliorative effect against lipid peroxidation probably due to the high antioxidant activity associated with its high phenolic content (Dada et al., 2017). The extract also enhanced the up-regulation of the activity of CAT. Treatment with STBI+RHEE did not SOD activity an enzyme required in the conversion of O^{2-} to the less reactive H_2O_2 and O_2 (Warner et al., 2004). Catalase (CAT) is important in helping the body to eliminate the H_2O_2 which is a by-product of O^{2-} metabolism thus enhancing antioxidant defense system (Warner et al., 2004; Adedara et al., 2018). Hence, RHEE demonstrated ameliorative effect on oxidative stress in both sharp and blunt TBI.

Haemorrhage in the motor cortex of the brain as demonstrated in the STBI and BTBI groups simulated cerebrovascular haemorrahagic injury which would affect motor coordination of the animal since corticospinal and corticobulbar tracts emanate from this area (Crossman and Neary, 2015). These are the tracts that modulate the voluntary skilled motor activities *via* the cranial and spinal nerves as well as

the cerebellum. The reduction in the size of the haemorrhage as shown in the slides of the STBI+RHEE and BTBI+RHEE groups when compared with those of STBI and BTBI respectively suggested that co-treatment with RHEE ameliorated the vascular damage done by the induced trauma. Literature reports have linked free radicals to the death of neurons and endothelial cells, as well as the altered contractile response of cerebral vessels as in subarachnoid haemorrhage (Ayer and Zhang, 2008). This was shown by the reduction in GSH level and antioxidant activities of SOD and CAT in the STBI and BTBI, whereas the increase in these parameters in the STBI+RHEE and BTBI+RHEE groups suggest modulation and minimizing of the vascular injury via antioxidative intervention by RHEE which might have led to the reduction of the vascular injury (among other probable factors), and amelioration of degenerated neurons in the histology.

Taken together, both sharp and blunt traumatic brain injury caused traumatic injury demonstrated by alterations in the body and brain weight, haematological, oxidative and histological parameters. Post-traumatic administration of RHEE ameliorated these changes possibly through its antioxidant property. This suggests that RHEE could be further investigated for possible identification of promising therapeutic agents against TBI effects.

REFERENCES

Adedara, I.A., Owoeye, O., Awogbindin, I.O., Ajayi, B,O., Rocha, J.B.T. and Farombi, E.O. (2018). Diphenyl diselenide abrogates chlorpyrifos-induced hypothalamic-pituitary-testicular axis impairment in rats. Biochemical and Biophysical Research Communications. 503(1): 171-176.

Algattas H. and Huang JH. (2014). Traumatic Brain Injury Pathophysiology and Treatments: Early, Intermediate, and Late Phases Post-Injury. Int J Mol Sci. 2014 Jan; 15(1): 309–341.

Arundine, M. and Tymianski, M. (2004). Molecular mechanisms of glutamate dependent neurodegeneration in ischemia and traumatic brain injury. Cell Mol Life Sci; 61: 657–68.

Ayer, R.E. and Zhang J. H. (2008). Oxidative stress in subarachnoid haemorrhage: significance in acute brain injury and vasospasm. Acta Neurochir Suppl. 104: 33–41

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K. Ezennia, E.C. and Atangbayila, T.O. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop J Pharmaceut Res. 7(3): 1019-1024.

Bancroft, J.D. and Gamble M. 2008. *Theory and Practice of Histology Techniques*, 6th edition. Churchill Livingstone Elsevier, Philadelphia; 83 - 134.

Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61:882–888.

Bramlett, H.M. and Dietrich, W.D. (2007). Progressive damage after brain and spinal cord injury: pathomechanisms and treatment strategies. Prog Brain Res.; 161:125–141.

Clark, R.S., Schiding, J.K., Kaczorowski, S.L., Marion, D.W. and Kochanek, P.M. (1994). Neutrophil accumulation after traumatic brain injury in rats: comparison of weight drop and controlled cortical impact models. J Neurotrauma; 11: 499–506.

Crossman, A.R. and Neary, D. (2015). Neuroanatomy. An Illustarted Colour Atlas Text. 5th Edition. Churchill Livingstone Elsevier, Edinburgh, pp 132-145.

Dada, F.A., Oyeleye, S.I., Ogunsuyi, O.B., Olasehinde, T.O., Adefegha, S.A., Oboh, G. and Boligon, A.A. (2017). Phenolic constituents and modulatory effects of Raffia palm leaf (Raphia hookeri) extract on carbohydrate hydrolyzing enzymes linked to type-2 diabetes. J Trad. Complem. Med. Available at: http://dx.doi.org/10.1016/j.jtcme.2017.01.003. Accessed August 03, 2017.

Davis, A.E. (2000) Mechanisms of traumatic brain injury: biomechanical, structural and cellular considerations. Crit Care Nurs Q; 23:1–13.

de Rivero Vaccari, J.P., Lotocki, G., Alonso, O.F., Bramlett, H.M., Dietrich, W.D. and Keane, R.W. (2009). Therapeutic neutralization of the NLRP1 inflammasome reduces the innate immune response and improves histopathology after traumatic brain injury. J Cereb Blood Flow Metab. 29(7):1251-1261.

Del-Maestro, R.F., McDonald, W. and Anderson, R. (1983). Superoxide Dismutase, Catalase and Glutathione Peroxidase in Experimental and Human Brain Tumours. In: Oxy Radicals and their Scavenger Systems, Greenwald, R. and G. Cohen (Ed.). Elsevier Publisher, New York. Vol. 2: 16-34.

Ebokaiwe, A.P., Adedara I.A., Owoeye O, and Farombi, E.O. (2013). Neurotoxicity of Nigerian bonny light crude oil in rats. Drug Chem Toxicol, 36 (2): 187-195.

Edem, D.O., Eka, O.U. and Ifon, E.T. (1984). Chemical evaluation of the nutritive value of the raffia palm fruit (Raphia hookeri) Food Chem. 15(1): 9-17.

Farran, A., Angoa-Perez, M., Briggs, D., Kuhn, D. and Esser, M.J. (2014) A Novel Model of Mild Traumatic Brain Injury for Juvenile Rats. *J. Vis. Exp.* (94), e51820, doi:10.3791/51820 (2014).

Feeney, D.M., Boyeson, M.G., Linn, R.T., Murray, H.M. and Dail, W.G. (1981): Responses to cortical

injury: Methodology and local effect of contusions in the rat Brain Res. 1981:211: 67-77

Gaetz, M. (2004). The neurophysiology of brain injury. Clin Neurophysiol**.** 115: 4-18.

Guimaraes, J.S., Freire, M.A.M., Lima, R.R., Souza-Rodrigues, R.D., Costa, A.M. and dos Santos, C.D. (2009). Mechanisms of secondary degeneration in the central nervous system during acute neural disorders and white matter damage. Rev Neurol; 48: 304–10.

Inci, S., Ozcan, O. and Kilinic, K. (1998). Time-level relationship for lipid peroxidation and the protective effect of tocopherol in experimental mild and severe brain injury. Neurosurgery 43:330–335.

Kriz, J. (2006) Inflammation in ischemic brain injury: timing is important. Crit. Rev. Neurobiol. 18,145–157.

Lenzlinger, P.M., Morganti-Kossmann, M.C., Laurer, H.L. and McIntosh, T.K. (2001). The duality of the inflammatory response to traumatic brain injury. Mol Neurobiol; 24: 169–81.

Maas, A.I., Stocchetti, N. and Bullock, R. (2008). Moderate and severe traumatic brain injury in adults. Lancet Neurol. 7:728–741.

Marklund, N., Bakshi, A., Castelbuono, D.J., Conte, V. and McIntosh, T.K. (2006) Evaluation of pharmacological treatment strategies in traumatic brain injury. Curr Pharm Des.12:1645–1680.

Mbaka, G.O., Ogbonnia, S.O., Olarewaju, O.T. and Duru, F.I. (2013). The effects of ethanol seed extract of Raphia hookeri (Palmaceae) on exogenous testosterone and estradiol induced benign prostatic hyperplasia in adult male rats. J. Morphol. Sci. 30(4): 235-243.

Mbaka, G.O., Ogbonnia, S.O., Oyeniran, K.J. and Awopetu, P.I. (2012). Effect of Raphia hookeri Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats, Brit J Med Med Res. 2(4): 621-635.

Ogbuagu, M.N. (2008). Vitamins, phytochemicals and toxic elements in the pulp and seed of raphia palm fruit (Raphia hookeri). Cirad/EDP Sciences. Fruits. 63: 297–302.

Ozdemir, D., Tugyan, K. Uysal, N. Sonmez, U. Sonmez, A. Acikgoz, O. Ozdemir, N. Duman, M. and Ozkan, H. (2005); Protective effect of melatonin against head trauma-induced hippocampal damage and spatial memory deficits in immature rats. Neurosci. Lett. 385: 234–239.

Park, E., Velumian, A.A. and Fehlings, M.G. (2004). The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma; 21: 754–4.

Public Health Service (PHS). (1996). Public health service policy on humane care and use of laboratory animals. US Department of Health and Human Services, Washington, DC, PL. 99-158.

Scholz, M., Cinatl, J., Schadel-Hopfner, M. and Windolf, J. (2007). Neutrophils and the blood-brain barrier dysfunction after trauma. Med Res Rev.; 27(3): 401-416.

Sinha, A.K. (1972). Colorimetric assay of catalase. Anal. Biochem. 47: 389-394.

Stelmasiak, Z., Dudkowska, K.A. and Rejdak K. (2000). Head trauma and neuroprotection. Med Sci Monit. 6(2): 426-432.

Szmydynger-Chodobska, J., Strazielle, N., Gandy, J.R., Keefe, T.H., Zink, B.J., Ghersi-Egea, J.F., and Chodobski, A. (2012). Posttraumatic invasion of monocytes across the blood-cerebrospinal fluid barrier. J Cereb Blood Flow Metab. 32 (1): 93-104.

Varshney, R. and Kale, R.K. (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol, 58(5): 733-43

Warner, D.S., Sheng, H. and Batinic-Haberle, I. (2004). Oxidants, antioxidants and the ischemic brain. J Exp Biol; 207: 3221–3231.

Weckbach, S., Neher, M., Losacco, J.T., Bolden, A.L., Kulik, L., Flierl, M.A., Scott E. Bell, S.E., Holers, V.M. and Stahel, PF. (2012). Challenging the role of adaptive immunity in neurotrauma: Rag1 (-/-) mice lacking mature B and T cells do not show neuroprotection after closed head injury. J Neurotrauma. 29(6):1233-1242.

Yamaura, I., Yone, K., Nakahara, S., Nagamine, T., Baba, H. and Uchida, K. (2002). Mechanism of destructive pathologic changes in the spinal cord under chronic mechanical compression. Spine; 27: 21–6.

Yilmaz, G. and Granger, D.N. (2008). Cell adhesion molecules and ischemic stroke. Neurol. Res. 30: 783–793.

Zhen-Guo, C., Guo-Dong, Z., Peng-Qiang, S. and Bao-Shun, D. (2013). Expression and antioxidation of Nrf2/ARE pathway in traumatic brain injury, Asian Pacific J Trop Med: 305-310.