

EFFECT OF MANNITOL AND URIC ACID ON LIPID PEROXIDATION AND LEVEL OF CREATINE KINASE IN ALBINO RATS INDUCED WITH TRAUMATIC BRAIN INJURY**¹Suleiman N., ^{2*}Bulama I., ³Suleiman M., ⁴Bilbis L. S. and ⁴Saidu Y.**¹Department of Veterinary Physiology and Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto.²Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri, Nigeria.³Department of Biochemistry, Bayero University Kanno,⁴Department of Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto,**Corresponding Author: Dr. Bulama I.**

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ABSTRACTS

Traumatic brain injury causes massive production of reactive oxygen species with resultant oxidative stress and impairment of endogenous antioxidant defense mechanisms. This study investigated the role of antioxidants in the management of traumatic brain injury. Traumatic brain injury was induced in 30 randomly selected wistar rats using weight drop method. The rats were divided into 6 groups of 5 rats each. Groups 1 & 2 were treated with 22.5mg/kg and 45mg/kg of mannitol while 3 & 4 were treated with same doses of uric acid orally for 14 days. Neurological assessment was done using Modified Glasgow coma scale. Blood and brain tissues were collected and analysis on lipid peroxidation and level of creatine kinase was conducted. The results showed that TBI significantly increased ($P < 0.05$) malondialdehyde concentration and creatine kinase activity. Supplementation with the antioxidants however reversed the trend in a dose dependent manner and decreased the mortality. In conclusion supplementation with mannitol and uric acid might have replenished the antioxidant defense system and have reduced oxidative lipid damage and therefore oxidative stress that is associated with increased mortality in TBI. This study highlighted the potentials for the use of antioxidants in the management of TBI.

KEYWORDS: Oxidative stress, Antioxidant, Glasgow coma scale.**INTRODUCTION**

Traumatic brain injury (TBI), also known as intracranial injury, occurs when an external force traumatically injures the brain. TBI can be classified based on severity, mechanism (closed or penetrating head injury), or other features (e.g. occurring in a specific location or over a widespread area).^[1] TBI is a major cause of disability worldwide, especially in children and young adults.^[2] Injury to the brain can be due to direct impact / acceleration or processes that are initiated after the impact.^[3] These processes, called secondary injury include excessive release of neurotransmitters, increase intracranial pressure, decreased cerebral blood flow, energy metabolism deficit, mitochondrial dysfunction, excessive release of reactive oxygen species and oxidative stress contribute substantially to the damage from the initial injury.^{[4],[5],[6]} The brain is the tissue most vulnerable to oxidative damage because of its high rate of oxidative metabolic activity, intensive production of reactive oxygen metabolites, relatively low antioxidant capacity, low repair mechanism activity, non-replicating nature of its neuronal cells, and the high lipid content.^{[7],[8]}

TBI is a leading cause of death and disability around the world and is associated with social, economic and health problems. The number of people with traumatic brain injury is difficult to assess accurately but it seems to be higher in the developing countries like Nigeria because of low adherence to safety standards and bad road network. Rehabilitation and management of the condition is the main treatment for serious conditions of TBI as there is no pharmacological therapy that has been approved for the treatment of TBI.^[11] Due to frequency of TBI, cost of management as well as lack of accepted therapy, it is imperative to search for affordable medications such as the use of antioxidants. This work is aimed at investigating the role of the antioxidants uric acid and mannitol in the management of traumatic brain injury in rats.

MATERIALS AND METHODS**Experimental animals**

Thirty apparently healthy albino rats of the wistar strain weighing between 180-200g were purchased from the Animal House of Bayero University, Kano, Nigeria. They were allowed to acclimatize for two weeks in the

laboratory. They were given clean water and feed ad libitum (vital growers feed).

Sources of chemicals

Uric Acid, Thiobarbituric acid, Trichloroacetic acid and n-butanol were all obtained from BDH Chemicals, Poole, England while Mannitol was obtained from Philip Harris Biological, Weston-super-Mare, England. Creatine Kinase assay kit (Lot No: - LIQ-576-A) was obtained from Chemelex, S.A, Barcelona, Spain.

Table 1; Design of the Experiment

GROUP	TREATMENT	NO OF RATS
I	Traumatized and Treated with 22.5 mg/kg Uric Acid	5
II	Traumatized and Treated with 45 mg/kg Uric Acid	5
III	Traumatized and Treated with 22.5 mg/kg Mannitol	5
IV	Traumatized and Treated with 45 mg/kg Mannitol	5
V	Traumatized non- treated	5
VI	Non-traumatized non- treated	5

INDUCTION OF TBI IN RATS

The rats were restrained with their heads lower than their bodies to avoid injuries to internal organs. The animals were anaesthetized with 3% isoflurane for duration of 5-10 minutes and not longer so as not to confer any form of neuroprotection by isoflurane. The skull was exposed by midline incision and a stainless steel disc measuring 10mm in diameter and 3mm in depth was cemented centrally along the position of the control suture between the lambda and bregma with a polyacrylamide adhesive. The animals were secured in a prone position on a 10 cm deep foam bed and injury was induced by dropping an 80g weight from a distance of 1m. The stainless steel disk was immediately removed from the skull.^[12]

Drug administration

Uric acid and mannitol to be administered were dissolved in normal saline and the volume to be administered was calculated using the formula below:-

$$\text{Volume} = \frac{\text{Body weight} \times \text{Dosage}}{\text{concentration}}$$

TISSUES PREPARATION FOR BIOCHEMICAL ANALYSIS

After decapitation, brains were immediately removed and dissected. Brain samples were taken from the frontal

Experimental Design

Thirty rats took part in the experiment; They were divided based on weight into 6 groups of 5 rats each as shown in the table below. The animals were treated once daily for a period of two weeks after the induction of trauma. All animals were monitored for recovery and survival from the day of trauma and through the period of recovery.

cortex and cerebellum.^[13] The brain samples were homogenized with Sorensen's phosphate buffer (pH 7.5) to mimic physiological conditions. Samples were stored in cold containers and carried to the laboratory immediately for analysis.

Estimation of biochemical parameters

MDA concentration was determined as an index to monitor lipid peroxidation, this was determined according to the method described by.^[14]

The activity of creatine kinase (CKBB) was estimated by the enzymatic method of.^[15]

STATISTICAL ANALYSIS

Results were expressed and presented as means \pm SD. Data were analysed by one-way analysis of variance (ANOVA), Duncans New Multiple Range Test was used for multiple comparison where significant difference exist among the mean values. Graphpad instat version 3.0 was used for the analysis.

RESULTS

The result in figure 1 shows that there was significant improvement in GCS of the groups treated with uric acid from the first week to second week of the treatment while the TNT group only showed slight improvement.

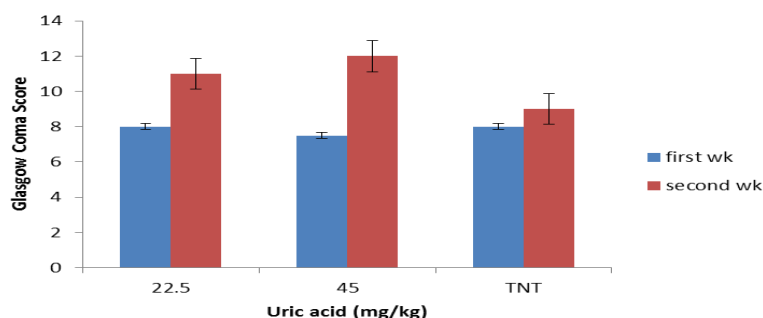


Figure 1: Glasgow Coma Score of Uric acid treated Groups

Figure 2 below represent GCS of rats administered mannitol in the first and second weeks of the treatment. from the result, rats treated with the 2 doses of mannitol

showed improvement on their GCS from average of 8 to 12 as the TNT rats slightly improved from average score of 8 to 9.

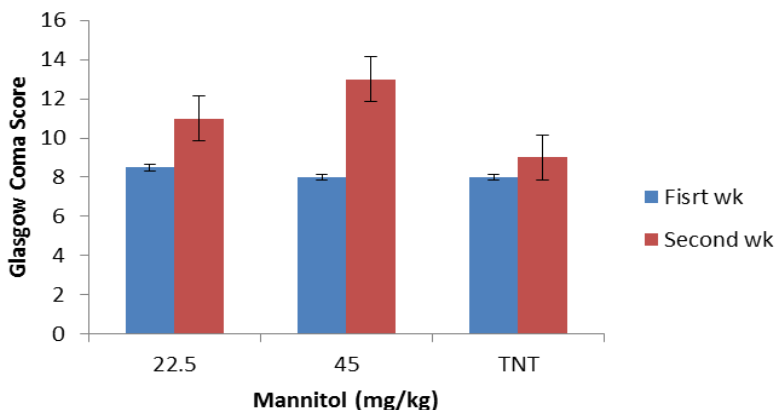


Figure 2: Glasgow Coma Score of Mannitol Treated Groups

Figure 3 presents the effect of treating experimental animals with uric acid on the creatine kinase activity. The level of the enzyme was observed to be significantly

($P < 0.05$) increased in the TNT group but it was significantly decreased in the groups Treated with two doses of Uric acid.

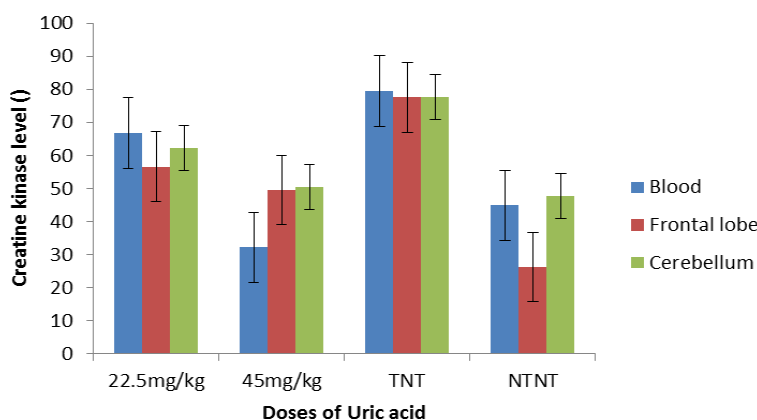


FIGURE 3: Effect of Uric acid on Creatine kinase Level in Blood and Brain of Experimental rats.

Figure 4 presents the effect of treating experimental animals with mannitol on the creatine kinase activity of various parts of the brain and blood. After trauma, the level of the enzyme was seen to be significantly ($P < 0.05$)

rised in the TNT group while treatment with different doses of mannitol caused significant decreased in the activity of the enzyme compared to the TNT group.

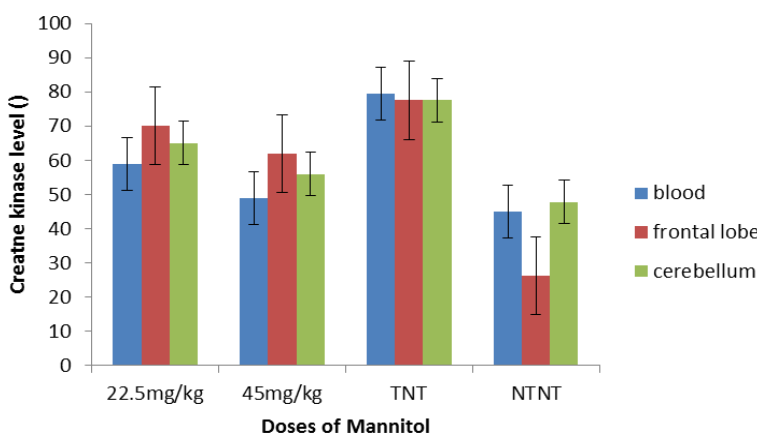


FIGURE 4: Effect of Mannitol on Creatine kinase Level in Blood and Brain of Experimental rats.

The effect of treatment with uric acid on MDA concentration is presented in Figure 5. The results indicated that TBI caused significant ($P<0.05$) increase in the concentration of MDA as seen in the TNT group.

Administration of uric acid at 22.5mg/kg and 45mg/kg BW significantly ($P<0.05$) decreased the concentration of MDA compared to the TNT group.

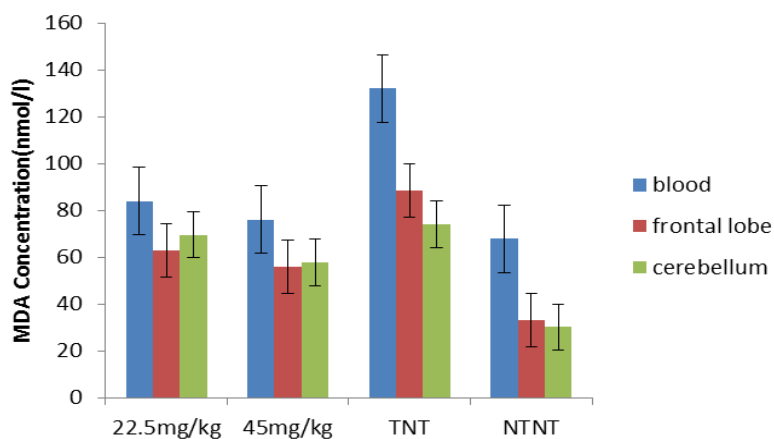


FIGURE 5: Effect of Uric acid on MDA Concentration in Blood and Brain of Experimental rats.

The effect of treatment with mannitol on MDA concentration is presented in Figure 6. The results indicated that there was significant ($P<0.05$) increase in the concentration of the MDA in the TNT group while

groups treated with mannitol at 22.5mg/kg and 45mg/kg BW showed significant decreased in the concentration of MDA compared to the concentration of MDA in TNT group.

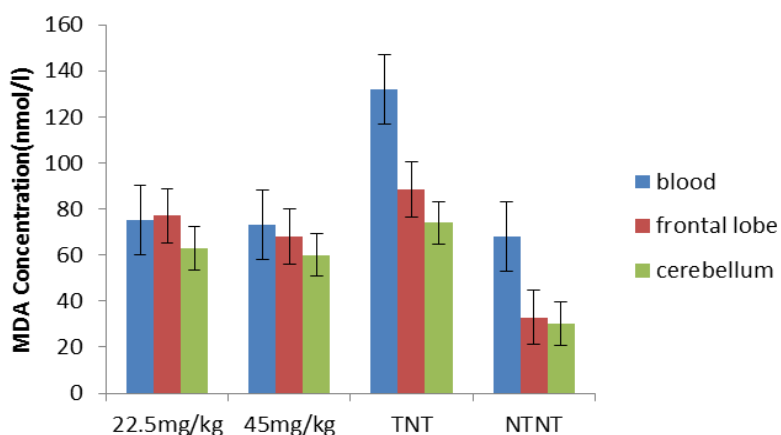


FIGURE 6: Effect of mannitol on MDA Concentration in Blood and Brain of Experimental rats.

DISCUSSION

Upon the induction of trauma, the signs of TBI were clearly evident. There was the obvious loss of consciousness, convulsions, urination and respiratory distress and which possibly results in apnea as reported by.^[13] Further evidences of trauma observed also include bleeding upon examination of traumatized animals, changes in behavior as the traumatized animals tend to pack together after recovery with little activity.

Traumatic brain injury induces spontaneous free radical generation leading to oxidative stress and consequent cell death.

The observed significant ($p<0.05$) increase in creatine kinase activity and concentration of MDA in all the

traumatized non treated rats compared to NTNT rats suggests a role of free radicals and oxidative stress. The result of the uric acid treated groups with 22.5 and 45mg/kg indicated significant dose dependent decrease in creatine kinase activity and MDA concentration. This is observed in both serum and brain tissue homogenate. This might be due to the ability of uric acid to physically scavenge free radicals such as superoxide, hydroxyl radical and singlet oxygen thereby reducing their oxidative damage. It can also be as a result of iron chelating effect of uric acid as it reduces the formation of more free radicals. This dose dependent effect of uric acid observed in this study is in agreement with the work of^[16] who reported concentration-dependent effect of uric acid in scavenging free radicals.^[17] also reported that Uric acid provides an antioxidant defense in humans.

Mannitol treatment of traumatized rats shows significant ($p < 0.05$) drop in the concentration of MDA and activity of creatine kinase, when compared with TNT rats in both serum and brain tissue. This is in line with the study of^[18], which shows that administration of mannitol increases the levels of the enzymes catalase and glutathione peroxidase, these enzymes would reduce the production of MDA which is a harmful substance for cells and thereby reduce cellular damage.^[19] reported that mannitol prevents formation of reactive oxygen species through the scavenging abilities of its hydroxyl groups, and this can probably be one of the reasons for the observed effect in this study.^[3] also reported similar effect of mannitol on MDA concentration following traumatic brain injury of rats.

CONCLUSION

Both uric acid and mannitol have proven to be effective at lowering creatine kinase activity and malondialdehyde concentration during traumatic brain injury. This indicated their usefulness in traumatic brain injury patients.

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