Antioxidant Level of Sodium Glutamate in Wister Rat whole Brain, Liver and Kidney from *Thevetia peruviana* kernel

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Abstract

The researchers examined the antioxidant level of sodium glutamate produced from Thevetia peruviana kernel in Wister rat whole brain, liver and kidney. The sample Thevetia peruviana kernel used in this study were obtained from Lafe area Akure, Ondo State, Nigeria and identified at the Department of Crop, Pest and Soil Management, Federal University of Technology, Akure. The samples were processed into three different techniques, namely: oven dried, air dried and sun dried. The processed samples were further ground to powder, packed in an airtight plastic sample and preserved in a refrigerator at 4ºC separately prior to the analysis. The production of sodium glutamate was done and the oven gave the best yield of sodium glutamate which was used for further analysis. A twenty (20) Wister rats were purchased and grouped according to their weight. The groupings (n = 4) were divided into five (5) in five baskets (1-5). The first basket serves as the control (0mg/kg) treated without the derived sample (sodium glutamate) and the remaining four were treated with the derived sample (sodium-glutamate) produced at different dosages 1 mg/kg, 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The anti-oxidant level (Reduced Glutathione (GSH) Activity in Tissue) of the Wister rats were carried out on the brain, liver and the kidney. From the result, it was discovered that sodium glutamate produced (seasoning) was safe as promising intervention in the management and prevention of oxidative stress, especially because of its direct antioxidative protecting ability and at the highest (1000 mg/ml) administered dosage, consequently indicating a bearable non-toxic effect on the organs of interest.

Keywords: Antioxidant, Sodium Glutamate, Wister Rat, Hepatic, Renal, *Thevetia Peruviana* Kernel

Introduction

Antioxidants play a critical role in reducing the free radicals' reactions in the body. The free radicals are chemical entities with one or more unpaired electrons. The free radicals

can cause changes in human body from DNA mutation to cell death. Such as change in DNA mutation, change in enzymatic activity, lipid peroxidation of cellular membrane and death (Birangane, Chole, Sathya & Shivaji, 2011). Low level of antioxidant enzymes indicates that free radical's level in the body is high (Durak, Calendar, Zun, Demir & Calendar, 2010). This can cause oxidative stress in human body.

Oxidative stress occurs when the amount of reactive oxygen species (ROS) in human body exceeds the amount of antioxidants in the body. This excess will attack the lipid, protein and DNA components of the body. Studies have confirmed that if the equilibrium between reactive oxygen species and antioxidants is slightly or strongly affected, oxidative stress will occur (Lobo, 2010; Hallwell & Gutharidge, 2015 and Rani, 2015).

Hee-young (2015) affirmed that antioxidant enzymes that catalyses oxidation production processing are able to serves as redox biomarkers in various human diseases and they are key regulator responsible for controlling the redox state of functional proteins. Alfadda Sallam & Kin (2012) described reactive oxygen species (ROS) as highly reactive molecules produced mainly by the mitochondria and from several byproducts of several cellular enzymes. Their work established that reactive oxygen species exert a broad spectrum of biological effect, ranging from physiological regulatory functions to damaging functions, confronting to several pathogenesis of diseases. Tsante (2006) referred physiological regulatory functions to damaging functions, confronting to several pathogenesis of diseases established that the reactive nature of ROS is high, thus, living organism must endeavour to maintain redox homeostatic under a well-regulated intricate system of antioxidant. The work of Miller *et al* (2013) and Saeidnia (2013) posed that redox imbalance can lead to oxidative stress reducing the potential therapeutic effect of the antioxidant.

Antioxidants mitigate the harmful effect of free radicals; these free radicals are molecules with one or more unpaired electrons (Zulaikhah, 2017). Hallwell & Gutteridge (2015) stated that side effect of free radical can increase with environmental factor. This will eliminate the reactive oxygen species in biological system. Zalaikhah (2017) maintained that low oxidants can be shown by high level of free radicals, he established that when the amount of free radicals exceed the amount of antioxidant in the body, the excess will attack the lipid, protein and DNA component. His work concluded that the major role of antioxidant is to stabilise the equilibrium between free radicals and biological system.

GSH determines the oxidative stress which can be induced due to excessive free radicals like ROS/RNS (reactive oxygen species or reactive nitrogen species) generated during metabolism of toxicants which may interact with cellular macromolecules such as DNA, lipids, proteins and thereby altering the structure and function of the cell. Oxidative stress occurs when there is an imbalance between radical activities, which may cause an increase in the formation of certain oxidation products (Schafer & Buettner, 2001). It was discovered by Akintelu & Amoo (2017) that *Thevetia peruviana* contained high amount of glutamate which is used for the production of seasoning. However, this work tends to investigate the antioxidant level of the seasoning (sodium-glutamate)

produced from *Thevetia peruviana* kernel and its effect on the whole brain, hepatic (liver) and the renal (Kidney) of a Wister rats.

Materials and Methods

Materials

Sample Collection

The *Thevetia peruviana* pulp samples used in this study were obtained from Lafe area Akure, Ondo State, Nigeria and identified at the Department of Crop, Pest and Soil Management, Federal University of Technology, Akure. The samples were processed into three different techniques, oven dried, air dried and sun dried. The processed samples were further ground to powder, packed in an airtight plastic sample and preserved in a refrigerator at 4ºC separately prior to the analysis.

Sample Preparation

Production of Monosodium-glutamate (seasoning) from *Thevetia peruviana* seed

The production of monosodium-glutamate from *Thevetia peruviana* seed was done using a natural fermentation. Natural fermentation is referred to wild or spontaneous fermentation where by the microorganisms are present naturally in the raw food or processing environment. In this study, a 100 g of each sample (sun, air and oven-dried) was weighed into 250 ml conical flask. A 150 ml of buffer (87 g of K_2HPO_4 and 68g of KH_2PO_4) was added to the samples, covered with foil paper and transferred into an electric shaker for 8 days at 37 ºC. After 8 days of fermentation, the samples were filtered using a clean white cloth to get the extract (liquid) and the residue. Subsequently, the extract (liquid) which is sodium-glutamate was freeze-dried to powder using the lyophilised machine. Similarly, 1 ml of the extract (liquid) sample was transferred into the centrifuge at 25 ºC and 4100 rpm for 30 min, its forms into two layers (the supernatant and the residue) and then the supernatant was poured into PET (polyethylene terephthalate) bottle for estimation of protein and glutamate determination using the *Ultraviolet*-visible spectrophotometer.

Reagent/Chemical/Apparatus Used

All the reagents and apparatus were of analytical grade as follows:

List of Reagents used

Tris- HCl buffer, Borine serum albumen BSA, Trichloroacetic acid, Sodium dodecyl sulphate, Ehrlich's reagent, Carbon teterachloride CCl_4, Wijs solution, Iso-propyl alcohol, Ellman reagent, Phosphate buffer, Calcium chloride $CaCl_2$, Mercury (II) chloride $HgCl_2$, n-butanol.

Methods

Animal grouping

A twenty (20) Wister rats were purchased and grouped according to their weight. The groupings (n = 4) were divided into five (5) in five baskets (1-5). The first basket serves

as the control (0mg/kg) without the derived sample (sodium-glutamate) and the remaining four were treated with the derived sample (sodium-glutamate) produced at different dosages 1 mg/kg, 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively.

Animal Treatment

After grouping the rats, they were acclimatised for four (4) days to familiarise with the new environment. The Wister rats were exposed for acute experiments and were treated with the derived sample (sodium-glutamate) for seven (7) days using a gavage at different dosage (1 mg/kg, 10 mg/kg, 100 mg/kg and 1000 mg/kg). There was also a group of rats that was used as control (without treatment).

Preparation of tissue homogenates of exposed rat samples for biochemical analyses

After the stipulated period of exposure of rat samples for acute experiment, the tissues of the rat samples namely; whole brain, hepatic (liver) and renal (kidney) tissues were quickly removed, weighed, placed on ice and homogenised (1:10 w/v) in cold 10 mM Tris-HCl pH 7.4. The homogenates were centrifuged at 4000 (CENTRIFUGE 80-2) for 10 min to yield the low-speed supernatant fraction that was used for the determination of thiobarbituric acid reactive substances (TBARS) and enzyme assays. For all analysis in this study, protein content was determined by the method of Lowry *et al* (1951), using bovine serum albumin (BSA) as the standard. The principle of the Lowry method depends on the reactivity of the peptide nitrogen with the copper ions under alkaline conditions and subsequent reduction of Follin reagent to heteropolymolybdenum blue by the copper-catalysed oxidation of aromatic amino acid.

Determination of Reduced Glutathione (GSH) Activity in Tissue

Reduced glutathione (GSH) was determined by the method of Ellman (1959).

Principle: Ellman reagent (5'5' dithio-bis-2-nitrobenzoic acid; DTNB) readily forms a mixed disulfide with thiols liberating the chromophore 5-mercapto-2-nitrobenzoic acid (absorption maximum at 412nm). Concentration of GSH is express as µmol/ g tissue.

The aliquots (200 µl) of tissue homogenate, 760 µl of 0.1M phosphate buffer (pH 7.4) and 10 µl of 0.1 mM DTNB {5, 5 dithio-bis (2-nitrobenzoic acid)} were mixed together and left in the dark for 10 mins. The reaction mixture was treated with 4% trichloroacetic acid in ratio 1:1 and centrifuged to precipitate the proteins. The amount of GSH was measured in the supernatant at 412 nm using a UV-visible spectrophotometer according to Ellman, (1959).

Result and Discussion

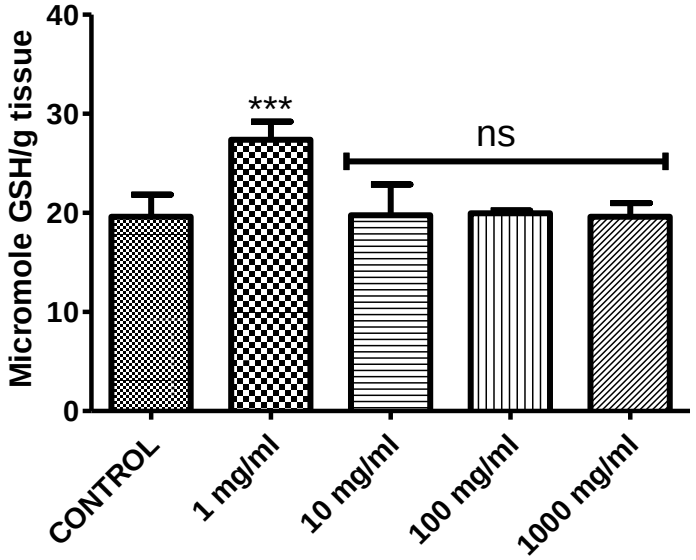

Figure1: Effect of sample on the level of protein thiol content in brain of treated Wister rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test (***) indicates degree of significance from control while (ns) indicates degree of non-significance from the control at $p<0.05$

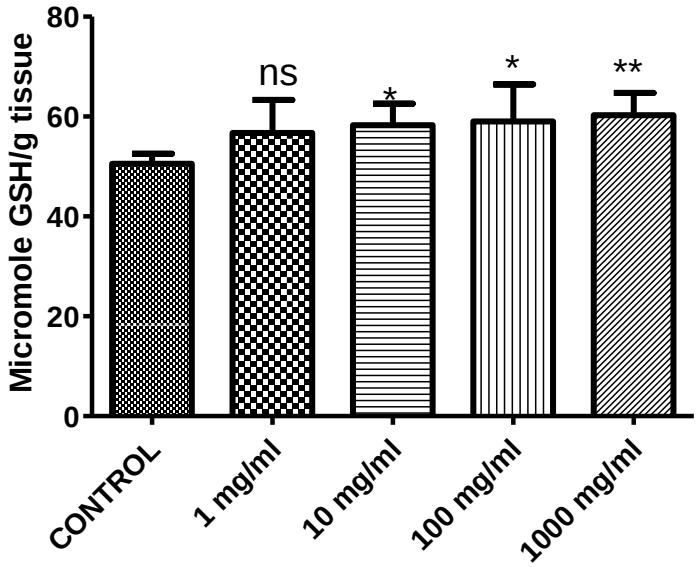

Figure 2: Effect of sample on the level of protein thiol content in liver of treated Wister rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test (***) indicates degree of significance from control while (ns) indicates degree of non-significance from the control at $p<0.05$.

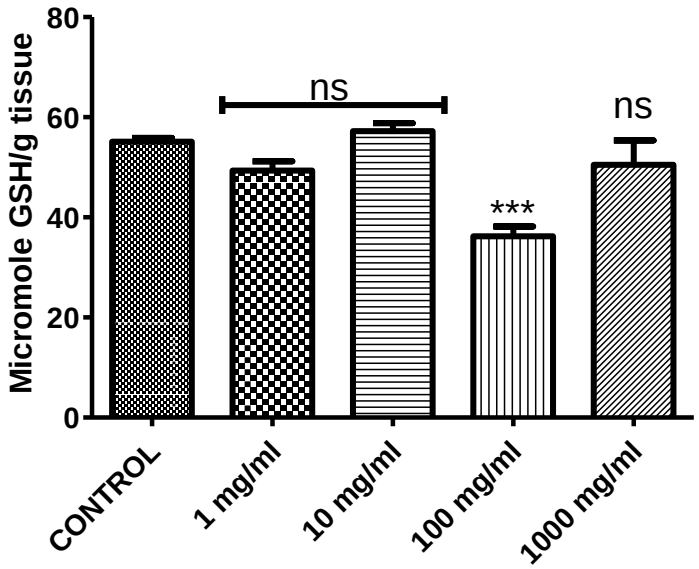

Figure 3: Effect of sample on the level of protein thiol content in kidney of treated Wister rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test (***) indicates degree of significance from control while (ns) indicates degree of non-significance from the control at $p<0.05$

Total Protein Thiol on the Brain, Liver and Kidney parts of Wister Rats

Drug-induced oxidative stress is implicated as a mechanism of toxicity in numerous tissues organ systems, including liver, kidney, ear and nervous systems. However, the extent to which mechanisms of drug-induced oxidative stress have been characterised varies. Metabolism of a drug may generate a reactive intermediate that can reduce molecular oxygen directly to generate ROS. The indices employed in the assessment of antioxidant status and oxidative stress toxicity of animals in the present study includes evaluation of the level total protein thiol and non-protein thiol in the brain, liver and kidney of the experimental animal, evaluation of ALA-D, NTPDase and TBARS. One-way ANOVA indicates that the extract exerted a significant positive influence on the antioxidant status of experimental animals generally. As presented in figure 1, 2 and 3, the extract treatment caused a marked increase and hence, the level of the total protein thiol in the brain, liver and kidney of the treated animals were not affected when compared to the control animals.

Previous research has revealed that in contrast to catalases and superoxide dismutases, which are well known endogenous antioxidants, peroxidases use a thiol-based reaction mechanism to detoxify hydroperoxides and their reduced state needs to be restored by the GSH/GR or the Trx/TrxR system under the consumption of NADPH (or in some special case NADH) (Ursini *et al* 1995). Peroxidases are set of endogenous antioxidant systems that have a very high affinity for peroxide (H_2O_2), based on their strictly conserved active site. The Thr/Cys/Arg triad facilitates the reduction of H_2O_2 by stabilising the transition state and polarising the O–O peroxyl bond, which promotes the

transfer of electrons from the catalytic cysteine (Nakamura *et al*., 2010; Hall *et al*., 2010; Rhee, 2016). This mechanism results in a reaction rate of 105-108 m^{-1} S^{-1} with H_2O_2 (Winterbourn & peskin, 2016), which is several orders of magnitude higher than the typical reaction rate of protein thiols with H_2O_2 (Winterbourn & Hampton, 2008; Marinho *et al* 2014). The sulfonic acid intermediate formed at the catalytic cysteine readily reacts in 2-Cys Prxs with the resolving cysteine to release water under the formation of a disulfide bond. The cycle is completed when the free thiol state is restored by the interaction with Trx-like proteins or other reductants like GSH. The effect of the extract on total protein thiol shown in this investigation is consistent with a recent study that found glutamate to be the preferred source of mucosal glutathione production in the rats. More so, glutamine supplementation decreases the levels of branched-chain amino acids under oxidative stress. This amino acid is a key metabolite associated with protein synthesis and cell growth (Ren *et al* 2014).

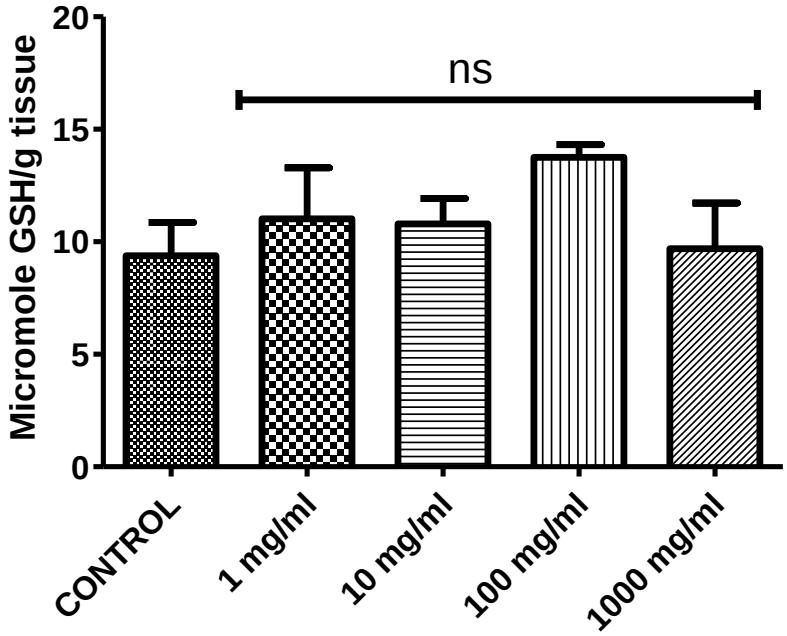

Figure 4: Effect of sample on the level of non-protein thiol content in brain of treated rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test. (ns) indicates degree of non-significance from the control at $p<0.05$

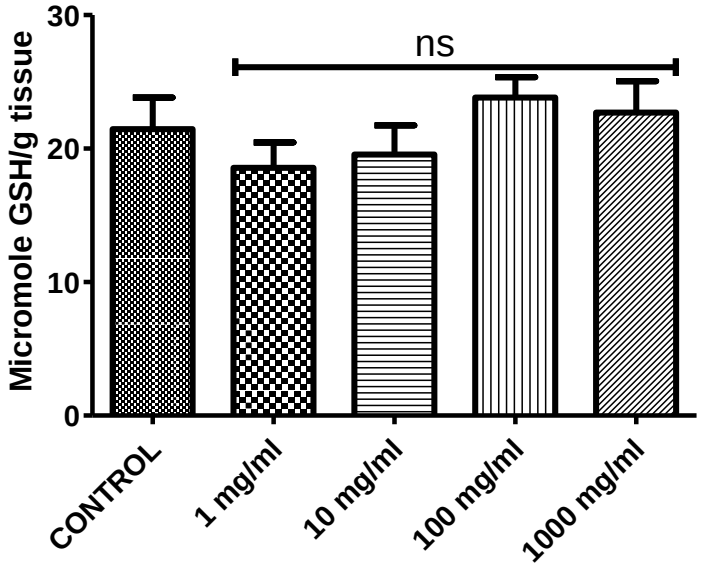

Figure 5: Effect of sample on the level of non-protein thiol content in liver of treated Wister rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test. (ns) indicates degree of non-significance from the control at $p<0.05$

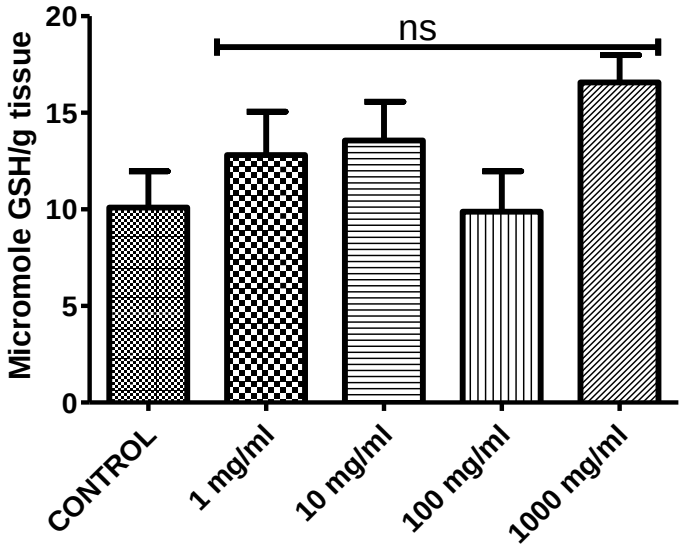

Figure 6: Effect of sample on the level of non-protein thiol content in kidney of treated Wister rats. Data are expressed as means ± SEM and were tested one-way ANOVA followed by Tukey's test (ns) indicates degree of non-significance from the control at $p<0.05$

Non-Protein Thiol on the Brain, Liver and Kidney parts of Wister Rats

The effect of the extract on non-protein thiol in the brain, liver and kidney is presented in figures 4, 5 and 6. These results revealed non-significant differences between the control and treatment group of animals across the varied doses in the brain, liver and kidney. Hence, from the present data which in part is confirmatory, it is logical to conclude that the sample utilised does not have toxic effect to the organs of interest. In most aerobic cells, the major non-protein thiol is glutathione (GSH); a tripeptide (gamma-Glu-Cys-Gly) that can be oxidised to its corresponding disulfide (GSSG). The intracellular concentration of GSH varies from 0.1 to 10 nM (Jones *et al* 2000).

The reduced redox state due to high intracellular concentrations of GSH is required to maintain protein thiols in a reduced state and to support a variety of redox reactions for reducing ROS, detoxifying xenobiotics and facilitating cell signaling. In addition, glutathione can directly prevent the oxidation of protein thiols by thiol-disulfide exchange and forming mixed disulfides. During the formation of mixed disulfides, the binding of GSH to protein thiols results in the addition of a glutathione molecule to the protein leading to the formation of glutathiolated or glutathionylated proteins. As a result of this investigation, it can be concluded that the extract has potential to therapeutically maintain the physiological level of GSH in the numerous organs of interest, which explains its non-toxic effect when compared to the control group.

Conclusion

In conclusion, based on the parameters evaluated, the extract from the sample has potential to therapeutically maintain the physiological level of GSH in the numerous organs of interest, which explains its non-toxic effect when compared to the control group and can also serve as source of anti-oxidant.

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