**ABSTRACT**

**Background:** Sodium nitroprusside (SNP) is an antihypertensive agent. It induces toxicity via the release of cyanide ions, nitric oxide (NO) and reactive oxygen species. *Manniophyton fulvum* (MF) is commonly used in Nigeria due to its therapeutic and nutritional potentials. This study evaluates the phytochemical composition of aqueous extract of MF root and influence in SNP induced oxidative stress in wistar rats.

**Methods:** Gas chromatography was used for determination of the chemical composition of aqueous extract of MF root. Rat liver homogenate was used for determination of rhodanese, glutathione (GSH) and malondialdehyde (MDA). Twenty (20) adult wistar rats of both sex were randomly divided into 4 different groups comprising 5 animals (n=5). Control (Group A), Groups B, C, D received 2.5 mg/kg body weight of SNP at intervals of 3 hours per day by intraperitoneal injection. In addition, Groups C and D received 200 mg/kg body weight of aqueous root extract of MF and 10 mg/kg body weight of Vitamin E respectively for a period of 7 days.

**Results:** Flavonoids had the highest composition while allicin had the lowest composition. Diallyl thiosulphinate>methyl allyl thiosulphinate >allyl methyl thiosulphinate. The activity of rhodanese, GSH and MDA concentrations showed that Group B had significant (p<0.05) increase in MDA concentration while GSH showed significant (p<0.05) decrease. Also, the activity of rhodanese showed significant (p<0.05) decrease compared to Group A. However, Groups C and D showed significant increase (p<0.05) in the activity of rhodanese enzyme compared to Group A and Group B. GSH levels of Group C and Group D showed no significant (p>0.05) difference while the MDA concentration showed significant (p<0.05) decrease. Correlation analysis between rhodanese and GSH showed strong significant (p=0.01, r=0.894) positive correlation.

**Conclusions:** From this study, it can be deduced that the chemical components of aqueous root extract of MF may serve as a pharmacological agent to up regulate detoxification and cytoprotective enzymes. Aqueous root extract of MF can also induced rhodanese to collaborate with GSH and promote inhibition of lipid peroxidation, anti-oxidative reactions and up regulate cyanide detoxification in tissues.

**Keywords:** SNP, MF, Oxidative stress, Rhodanese, Glutathione
to exhibit anti-oxidative functions and maintain redox homeostasis in mammals. Covalent modifications of sulphydryl groups by sulfane sulphur regulate many cellular processes. These reactions are mostly catalyzed by sulfur transerases, that catalyze the transfer of sulfane sulfur to suitable acceptor without cofactor. Example of such enzyme is rhodanese (thiosulfate: cyanide sulfur transferases; E.C. 2.8.1.1). Rhodanese is an enzyme that is found in many organisms. Intracellular studies had indicated the presence of this enzyme in cytosol, mitochondria and nucleus. The primary biological function of rhodanese is to catalyze the detoxification of cyanide via transfer of the sulfane sulfur from thiosulfate to cyanide and to generate thiocyanate and sulfite which are less toxic. Recently, the enzyme has been described as a multifunctional enzyme owing to its ubiquitous nature and variety of functional domain. It has been involved in many cellular functions such as supply of sulfur for iron-sulphur centers, biosynthesis of thiamine/molybdenum cofactors, selenium metabolism, thioredoxin oxidation (as thioredoxin oxidase). The role of rhodanese in the mitochondrial sulfide oxidation pathway has also been discovered to catalyze the transfer of sulfane sulfur from glutathione persulfide (GSSH) to sulfite to form thiosulfate, a substrate for rhodanese. MF belongs to the Euphorbiaceae family. It is commonly used in Nigeria because of its therapeutic and nutritional potentials. Different parts of this plant have been used to treat illnesses like cough, dysentery, hemorrhoids, erectile dysfunction, diarrhea etc. The leaves of MF have investigated to exhibit hypoglycemic and antioxidant effects. Its aqueous root extract administered to wistar rats exposure to bonny light crude oil was also reported to restore imbalances in glucose metabolism. Thus, the study seeks to investigate the chemical composition of aqueous extract of *Manniphyton fulvum* (MF) roots and effects in SNP induced oxidative stress in wistar rats in vivo by evaluating rhodanese enzyme activity, Glutathione (GSH) and malondialdehyde (MDA).

**METHODS**

**Collection and identification of plant material**

Roots of MF were collected from Umuada Ngodo Isuochi, Umunneochi Local Government Area of Abia State, Nigeria. The roots were identified at the Herbarium of University of Port Harcourt, Nigeria.

**Reagents and chemicals**

SNP (25 mg/ml) was obtained from Llydo’s Pharmacy along Agip Road, Port Harcourt by Biopharma Pvt Ltd, Navi Mumbai, Indian. KH$_2$PO$_4$, K$_2$HPO$_4$, Ellmans reagent and thiobarbituric acid were from Sigma Aldrich (St. Louis, USA). All other reagents and chemical were of analytical grade supplied by the Department of Biochemistry, Rivers State University, Nigeria.

**Preparation of root extracts**

The roots were sorted, cleaned and air dried at room temperature for two weeks. The dried roots were crushed into coarse powder with grinding machine and weighed. 200 g of the coarse powder was directly soaked in 1000 ml of hot water for 72 hours with intermittent shaking to facilitate extraction at room temperature. The extract was then filtered using a Buchner funnel and Whatman no. 1 filter paper and evaporated to dryness using rotary evaporator oven dried at a temperature of 50°C and later reconstituted in separately in 0.9% normal saline and dose administered according to the body weight of animals.

**Phytochemical quantification**

Phytochemical quantification of the aqueous root extract was done using gas chromatography (GC) (Hewlett Packard, Wilmington, DE, USA), powered with HP chemstation Rev. A09:01 (10206) software. Phytochemicals were identified and quantified by its interaction with the column stationary phase. The GC was programmed as follows: the inlet and injection temperature was set at 250°C– 310°C; fused silica column [30 m x 0.25 mmx0.01 μm thickness]; split injection was adopted with a split ratio of 20:1 and volume injected was 1 μL. Oven temperature was set at 60°C for 5 mins initially, heated to 65-260°C at 12°C/min; 260-320°C at 15°C/min and maintained at 310°C for 18 min. Nitrogen was used as carrier gas and pressure was 30 psi. Comparison of retention times and spectral data of standard were used for identification of phytochemicals. Standard calibration curve for each component was used for quantification.

**Experimental animals**

20 adult wistar rats of both sex weighing 120-150 g were used for this study. They were obtained from the animal house of University of Port Harcourt, taken to the animal house of the Department of Biochemistry, Rivers State University, Nigeria. They were allowed to acclimatize under laboratory conditions at room temperature of 25±2°C, a 12 hours hour light and dark cycle with 50±5% humidity for seven days. Food and water were given ad libitum prior to the conduct of the experiment. The experiment was conducted in accordance with the University’s ethical guidelines for use of laboratory animals.

**Induction of oxidative stress**

SNP was freshly prepared in 0.9% sterile saline on each day for administration. 2.5 mg/kg body weight of SNP was administered at intervals of three hours per day to rats that were fasted overnight by intraperitoneal injection for a period of 7 days according to body weight. At the end of administration, the animals were sacrificed by decapitation and livers excised for biochemical investigation.
Table 1: Grouping of the animals and administration protocol.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Treatment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group A (Control)</td>
<td>Received 1 ml of 0.9% saline</td>
</tr>
<tr>
<td>2</td>
<td>Group B</td>
<td>Received 2.5 mg/kg body weight of SNP only</td>
</tr>
<tr>
<td>3</td>
<td>Group C</td>
<td>Received 2.5 mg/kg bodyweight of SNP and 200 mg/kg body MF</td>
</tr>
<tr>
<td>4</td>
<td>Group D</td>
<td>Received 10 mg/kg body weight of Vitamin E</td>
</tr>
</tbody>
</table>

Grouping of the animals and administration protocol

Animals were randomly divided into four different groups comprising five animals in each group (n=5). The first was the control and the others were the test groups. 200 mg/kg body weight of the extract was administered orally for a period of seven days. Administration protocol of drug and plant extract is presented in Table 1.

Preparation of liver homogenates

The rat livers were washed with ice-cold saline solution (0.9% w/v), blotted, and weighed. Liver homogenate was prepared by homogenizing 1 g of the tissue in 5 ml of 0.05 M phosphate buffer, pH 7.4 using Teflon coated homogenizer. The homogenate was centrifuged for 10 minutes at 10000g, the sediment discarded and supernatant used for the assay of rhodanese, GSH and MDA.

Determination of biochemical parameters

Rhodanese enzyme

The activity of rhodanese in the sample was estimated spectrophotometrically. Thiocyanate produced by the action of the enzyme on cyanide and thioulsinate, is reacted with ferric nitrate reagent to produce a red colored complex- ferric thiocyanate. The absorbance of this complex is measured at 460 nm. Briefly, the assay mixture contains 10 μl of the supernatant (enzyme source) was incubated with 0.2 ml of 0.24 M potassium cyanide, 0.2 ml of 0.05 M phosphate buffer and 0.2 ml of 0.25 M sodium thiocytate pentahydrate for 20 min. The incubations were terminated by the addition of 0.5 ml of 15% (v/v) formaldehyde. The amount of ferric thiocyanate formed by the addition of 1.5 ml ferric nitrate reagent (10 g of ferric nitrate nonahydrate and 20 ml of 65% nitric acid per 150 ml) was read at 460 nm. The enzyme activity was expressed as amount of ferric thiocyanate formed per min per mg of protein for liver preparations.\textsuperscript{11-12} Protein concentrations were determined by the method of using crystalline bovine serum albumin as standard.\textsuperscript{13}

GSH

GSH was estimated by the method of Ellman’s reagent. The method involves the development of a yellow color using Ellman’s reagent. The absorbance of the color developed after reaction is read at 412 nm. The assay mixture contains 0.1 ml of the homogenate and distilled water, 2.5 ml of EDTA and 1.3 ml of TCA and centrifuged at 10,000g for 10 minutes to obtain the supernatant. 2 ml of Ellman’s reagent 5,5'-dithiobis-(2-nitrobenzoic acid) and 1.5 ml of phosphate buffer were added to 1.0 ml of the supernatant. The absorbance of the resulting solution was measured at 412 nm wavelength; a set of standard solution containing reduced glutathione (10 mg/100 ml) was prepared and read accordingly. The result was expressed as μmol of GSH/mg protein.\textsuperscript{14}

Lipid peroxidation

Lipid peroxidation (LPO) product was determined as MDA. In this method, the samples are heated with thiobarbituric acid reagent for 20 mins in a boiling water bath to give a pink colored complex that is read at 535 nm wavelengths. 1.0 ml of the liver homogenate, 1.3 ml of TCA, 2.45 ml of EDTA and 1.2 ml of normal saline was added and mixed thoroughly in a tube and centrifuged at 3000 g for 10 mins. 1.5 ml of distilled water and thiobarbituric acid were added to 1ml of the supernatant which was boiled for 20 mins. The pink color produced was measured at 535 nm against a blank. MDA equivalent in sample was estimated using the extinction coefficient of 1.56 × 10\textsuperscript{5} M\textsuperscript{-1}cm\textsuperscript{-1} and expressed as nmol (MDA) per mg protein.\textsuperscript{15}

Statistical analysis of data

Data obtained from this study were expressed as mean±standard error of mean (SEM). One-way analysis of the variance and turkey post hoc test was used for the establishment of significance differences set at (p<0.05). P values for correlations were derived from the Pearson correlation coefficient (r).

RESULTS

The result obtained for the phytochemical investigation of the aqueous root extracts of MF is shown in Table 2. Phytochemicals like allicin, carotenoids, flavonoids, phytosterols, glycosides and saponins were identified and quantified. Flavonoids had the highest composition (67.48%) while allicin had the lowest composition (0.0003%). Similar studies conducted on the ethanol and aqueous leaf extracts revealed that flavonoids had the highest composition in both extracts. In Table 3, the result showed that diallyl thiosulphinate had the highest composition (95.91×10\textsuperscript{-6}%), followed by methyl allyl thiosulphinate (3.50×10\textsuperscript{-6}%) and then allyl methyl...
thiosulphinate (0.58×10^{-8}g). The activity of rhodanese, GSH and MDA determined in the rat liver homogenate of SNP-induced oxidative stress is shown in Table 4. The result showed that Group B which was administered with SNP only showed significant (p<0.05) increase in MDA concentration while GSH showed significant (p<0.05) decrease also, rhodanese showed significant (p<0.05) decrease compared to the control (Group A). However, Groups C and D showed significant increase (p<0.05) in the activity of rhodanese enzyme compared to Group A and Group B. GSH levels of Group C and Group D showed no significant (p>0.05) difference while the MDA concentration showed significant (p<0.05) decrease. The decrease observed in rhodanese activity and GSH in Group B may be attributed to depletion of reactive sulfur species due to participation in cyanide detoxification and antioxidant activity. In Groups C and D increase in rhodanese enzyme activity may be attributed to the role of MF to maintain sulphur pool, enhance the conversion of cyanide to thiocyanate and provide protection against the toxicity of cyanide and oxidation induced by CN and NO.

Correlation analysis carried out to assess the relationship between the activity of rhodanese and GSH is presented in Figure 1, the result showed strong significant (p=0.01, r=0.894) positive correlation between Group C and Group D. This is an indication of the extract to enhance antioxidant activity.

**DISCUSSION**

Plants produce different bioactive compounds or phytochemicals that act as defense and regulate some physiological processes in man which include anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, anti-inflammatory, endothelial function enhancement, antioxidant activity, hepatoprotection, nephroprotection etc. Bioactive components like allicin, carotenoids, flavonoids, phytosterols, glycosides and saponins were identified and quantified in the aqueous extract of MF root. Understanding the chemical interactions of these bioactive components and the organisms can provide pharmacological agents and their molecular targets to address chronic diseases in biological systems. Flavonoids had the highest composition while allicin had the lowest composition. Similar studies conducted on the ethanol and aqueous leaf extracts revealed that flavonoids had the highest composition in both extracts. Components of allicin (organosulfur compounds), diallyl-thiosulfinate is one of the major organosulfur compounds considered to be biologically active. These compounds exhibit intriguing biological properties such as antimicrobial, antioxidant and anti-inflammatory. They are able to up-regulate detoxification and cytoprotective enzymes. In vivo demonstration has also shown that diallyl disulfide, occurring in garlic, elevated hepatic sulfane sulfur level and activities of gamma-cystathionase and 3-mercaptopypyruvate sulfur transferase in tumor bearing mice indicating hepatoprotection.
SNP, chemically known as SNP dihydrate (Na$_3$[Fe(CN)$_5$NO]$_2$H$_2$O), a salt of ferric cyanide containing nitric acid with five CN molecules. It exhibits fast-acting vasodilatory properties thus, used to control hypertension and bleeding during cardiac surgery. The metabolism of the drug has been widely studied both in experimental animals and humans as classical inductor of oxidative stress. From the study, the group administered with SNP only showed significant increase in MDA concentration while rhodanese and GSH activity showed significant decrease compared to the control which may be attributed to the decomposition of SNP. The decrease observed in rhodanese activity and GSH may be attributed to participation in cyanide detoxification and antioxidant activity. When SNP is administered, CN ions are released non-enzymatically and react with haemoglobin in blood and tissue sulphhydryl groups resulting to the release of other oxidative stress mediators which include NO and iron ions. High concentrations of NO are toxic and interact with superoxide anion (O$_{2^-}$) to form peroxynitrite (ONOO$^-$). ONOO$^-$ is protonated to form peroxynitrous acid (HOONO), at physiological pH, decomposes further to another potent oxidant that could initiate LPO. In the blood, the drug changes oxyhaemoglobin to methemoglobin and cyanide has a high affinity for this type of methemoglobin. One free CN$^-$ reacts with it to form cyanomethaemoglobin and sequesters cyanide instead of oxygen to the body. The remaining four free CN are transported to the hepatocyte, react with thiosulfate and cobalamin to form thiocyanate and excreted by the kidneys. Those not eliminated bind to both ferric (Fe$^{3+}$) and ferrous (Fe$^{2+}$) in tissue cytochrome oxidase inhibiting oxidative phosphorylation and concomitant production of ATP, the energy currency of cell. Alternative energy production leads to abnormal production of lactic acid that may result to severe lactic acidosis. Groups C and D showed significant increase in the activity of rhodanese enzyme and GSH and decrease in MDA concentration compared to Groups A and B suggesting a role of MF to enhance the conversion of cyanide to thiocyanate, intra mitochondrial oxygen free radical detoxification and protection against oxidative stress induced by SNP in the rats. A study on the effects of Allium cepa on rhodanese activity during cyanide toxicity showed that rhodanese activity was more enhanced in the presence of essential oil of onion than Na$_2$S$_2$O$_3$. The increased activity of rhodanese and GSH may also be attributed to collaborative function in which GSH serve as a substrate for the enzyme. Kinetic data combined with simulations at physiologically relevant substrate concentrations have established that the first enzyme in the mitochondrial sulfide oxidation pathway (sulfide quinone oxidoreductase) catalyzes the synthesis of GSSH, which is a substrate for rhodanese. GSH act as an antioxidant and control ROS production during oxidative stress and maintain GSH homeostasis for cellular redox balance. The participation of rhodanese in the oxidative stress response has been investigated in studies conducted by comparing activity of bovine rhodanese isoforms in sulfur acceptor from physiological donor substrate.

**CONCLUSION**

Cell-redox modulation has recently received increased attention because of many plant products that can attenuate harmful biological reaction. The chemical components of MF had indicated the presence of organosulphur derivatives especially those bearing the more reactive “allyl” rather than a “propyl” moiety and may serve as a pharmacological agent to up regulate detoxification and cytoprotective enzymes. Cellular localization of rhodanese enzyme was also induced by aqueous extract of MF root in collaboration with GSH and promote inhibition of lipid peroxidation, anti-oxidative reactions and up regulate cyanide detoxification in tissues.

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**Conflict of interest: None declared**

**Ethical approval: The study was approved by the Institutional Ethics Committee**

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