Changes in Nitric Oxide Levels in Lead-Exposed Male Rats Co-Treated with Methanolic Extracts of *Curcuma longa* Rhizomes and *Spondias mombin* Leaves

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Abstract*—Nitric oxide (NO) is a known vasodilator compound and in humans and animals, NO is a signaling molecule that play vital role in many physiological and pathological processes. Considering the vital biological roles of NO, this study evaluated the possible changes in nitric oxide levels in lead-exposed male rats co-treated with methanolic extracts of some local spice and herb (*Curcuma longa* rhizomes and *Spondias mombin* leaves (MECL and MESM respectively)). Fifty four (54) adult male wistar rats weighing between 150g and 200g were procured for the study and divided into nine (9) groups including Group 1: Control (rats received 1ml of distilled water and normal feed daily); Group 2: received 100mg/kg lead acetate in drinking water throughout the study period; Group 3: rats received 100mg/kg lead acetate in drinking water + Vitamin E (100mg) dialy; Group 4: rats received 100mg/kg lead acetate in drinking water + 500mg/kg MECL; Group 5: rats received 100mg/kg lead acetate in drinking water + 1000mg/kg MECL; Group 6: rats received 100mg/kg lead acetate in drinking water + 200mg/kg MESM; Group 7: Rats received 100mg/kg lead acetate in drinking water + 400mg/kg MESM; Group 8: Rats received 100mg/kg lead acetate in drinking water + 200mg/kg MESM+500mg/kg MECL and Group 9 Rats received 100mg/kg lead acetate in drinking water + 400mg/kg MESM+1000mg/kg MECL. Treatments were done daily for 4 weeks. The outcome of the present study revealed that NO levels in groups 3, 6 and 9 were significantly ($P<0.05$) higher when compared to group 2 (Pb treated only). In conclusion, the marked elevation of serum NO level following the co-administration of the methanolic *Curcuma longa* rhizomes and *Spondias mombin* leaves reveal that they may possess therapeutic potentials in stabilizing nitric oxide deficiency.

***Keywords*: Nitric oxide (NO); *Curcuma longa*; *Spondias mombin* leaves; therapeutic potentials; lead acetate**

I. INTRODUCTION

Nitric oxide (NO) is a known vasodilator compound in humans and animals; it is a signaling molecule that plays vital roles in many physiological and pathological processes [1]. Typical pharmaceuticals, for example, nitroglycerine and amyl nitrite are precursors to nitric oxide. Due to its significance in neuroscience, general physiology, and immunology, NO was declared "Particle of the Year" in 1992 [2]. Investigation into its relevance prompted the 1998 Nobel Prize for explaining the functions of nitric oxide as a major cardiovascular system regulatory molecule [1].

Dietary nitrate too is a key source of NO in both humans and animals. Some green vegetables and some root vegetables have high concentrations of nitrate [3]. When absorbed in the body, nitrate gets concentrated in saliva and is subsequently converted to nitrite on top of the tongue through a bio-film of commensal facultative-anaerobic bacteria [4]. When the saliva is swallowed, in the stomach, the nitrite reacts with hydrochloric acid and reducing agents (like ascorbate) to produce high concentrations of nitric oxide [5].

The nitrate-nitrite-nitric oxide pathway increases NO via sequential reduction of dietary nitrate derived from plant-based foods. Nitrate-rich vegetables have been said to elevate cardioprotective levels of nitric oxide with a resultant reduction in blood pressure in pre-hypertensive persons [6], [7].

Globally, there is a trend towards finding solutions to numerous ailments using herbal medicines and products. Recently, researchers have also begun to discover the antioxidant effect of these plants. To support these efforts, the World Health Organization (WHO) has proposed that the use of herbs is safe and could be an alternative to synthetic drugs in many disease conditions [8]. Recent discoveries of new infectious diseases, the proliferation of non-communicable diseases (NCDs) such as hypertension, diabetes, cancer, etc., their increasing multidrug resistance and accompanying side effects, have prompted renewed researches in treatment with potential drug molecules from herbs [9]. *Curcuma longa rhizome* and *Spondias mombin leaf* are typical examples of such plants that have been said to ameliorate several diseases due to reports of their possible rich phytochemical contents which include several antioxidants [10], [11].

Curcuma longa, a plant from the ginger family, Zingaberaceae is a shrub that measures up to 1 m high with a short stem, It can be found all over tropical and subtropical

regions of the world; predominantly cultivated in Asian countries, principally in India and China [12]. It is generally called Turmeric [2].

Tumeric is the yellowish powdered form of *Curcuma longa* rhizome. It is used to spice up food, as food preservative and also to produce some food colouring agents like curry powder. Tumeric has been proven by various researchers and traditional medicine practitioners to be beneficial for the treatment of hypertension, diabetes, cough, including other oxidative stress related disease [10].

Spondias mombin (S. mombin), commonly known as Hog plum tree is also widely cultivated in the tropical forest zones like Nigeria, Brazil, etc [13]. *S. mombin* Linn (Anacardiacea) is a valuable plant with multiple potentials and untapped resources. The leaves, fruits and bark are edible and proven to possess high antioxidants. Its leaf has antimalarial, antimicrobial, anti-inflammatary, antihypertensive effects [11], [14], [15].

Considering the vital physiological and pathophysiological roles of nitric oxide and its levels, the present study set out to evaluate the possible NO level regulatory properties of both combined and separate treatments with methanolic extracts of *Curcuma longa* rhizome and *Spondias mombin* leaves in male wistar rats.

II. MATERIALS AND METHOD

This study was conducted in the animal house of the Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt and lasted for a period of six (6) weeks. Prior to the commencement of the work ethical approval was sought and obtained from the institution's Central Ethics Committee.

A. Collection, Identification and Preparation of Plant Materials

Fresh leaves of *S. mombin* were collected from the gardens in the premises of the University of Port Harcourt. Fresh *Curcuma longa* rhizomes were purchased from a local market, Fruit-Garden Market, Kaduna Street, Port Harcourt, Rivers State, Nigeria. Subsequently, they were identified, authenticated and voucher number issued (UPH/P/165 for *Curcuma longa* and UPH/P/166) for *Sponias mombin)* by a plant taxonomist, Ekeke, C. (PhD), of the University of Port Harcourt Herbarium, University of Port Harcourt, Choba, Rivers State, Nigeria.

B. Preparation of Extracts for Spondias mombin leaf and Curcuma longa rhizome

S. mombin leafs and *C.longa* rhizomes were separately washed and air dried and then ground to fine powder using a motorized electric grinder. About 1.4 kg and 100g of the powdered forms of *S. mombin* leafs and *C.longa* rhizome respectively were separately soaked with 3.5 L and 0.5L respectively of analytical grade methanol in covered jars [16]. The content of the jars were macerated intermittently for proper mixing and left to stand for about 72 hours each. The separate mixtures were filtered and concentrated using a rotary evaporator at 45^0C. The separate semi-liquid extracts of the two plants were labeled and stored at a temperature below 4°C in a refrigerator until the time of administration.

C. Procurement of Experimental Animals

Fifty four (54) adult male wistar rats weighing between 150g and 200g were procured from the Animal House of the Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State. The animals were housed in wooden/wire-gauzed cages and were allowed access to standard rat chow and clean tap water *ad libitum* while allowing them to acclimatize for 14days before grouping them and commencement of treatments on them.

D. Induction of Experimental Nitric oxide deficiency

Nitric oxide deficiency was experimentally induced by oral administration of 100mg/kg of lead acetate in drinking water throughout the six week duration of the main experiment [17]. Confirmation of experimentally induced nitric oxide deficiency was made after two weeks of commencement of lead acetate. Afterwards, treatment with the extracts commenced.

E. Treatment Protocol for the Study Animals

There were nine (9) groups of six (6) rats each weighing between 150g-200g

i. **Group 1:** Control (rats received 1ml of distilled water and normal feed daily).
ii. **Group 2:** lead acetate (Pb $(NO_3)_2$) only treated rats (in addition to normal feed and 100mg/kg of Pb $(NO_3)_2$ contaminated water *ad libitum* throughout the study period.
iii. **Group 3:** Rats were treated (per oral) with 100 mg/kg lead acetate and synthetic antioxidant drugs-Vitamin E (100mg) for 4 weeks
iv. **Group 4:** Rats had oral administration of 100 mg/kg of lead acetate in drinking water with a low dose (500mg/kg) of *Curcuma* extract for 4 weeks.
v. **Group 5:** Rats received 100mg/kg of lead acetate in drinking water and a high dose (1000mg/kg) of *Curcuma* extract for 4 weeks.
vi. **Group 6:** Rats had oral administration of 100mg/kg lead acetate in drinking water and a low dose (200mg/kg) *Spondias mombin leaf* extract for 4 weeks.
vii. **Group 7:** Rats were given 100mg/kg of lead acetate in drinking water orally and a high dose (400mg/kg) *Spondias mombin leaf* extract for 4 weeks.
viii. **Group 8:** Rats had oral administration of 100mg/kg lead acetate in drinking water and co-administration with 500mg/kg *Curcuma* and 200mg/kg *Spondias mombin* leaf extract.
ix. **Group 9:** Rats were given 100mg/kg lead acetate in drinking water and co-administration with

1000mg/kg *Curcuma* and 400mg/kg *S. mombin* leaf extract.

F. *Determination of Nitric oxide (NO) Level*

The nitric oxide levels in the serum of the experimental rats were determined using the Griess reagent method as stated in [18] and the values were expressed in µmol/ml.

G. *Statistical Analysis*

The quantitative data generated from this study were analyzed using Analysis of Variance (ANOVA). Values were reported as Mean ± standard error of mean (SEM). Significant difference across the mean values of the various groups was determined at 95% confidence level with p-value < 0.05 using SPSS (statistical package for social sciences).

III. RESULTS

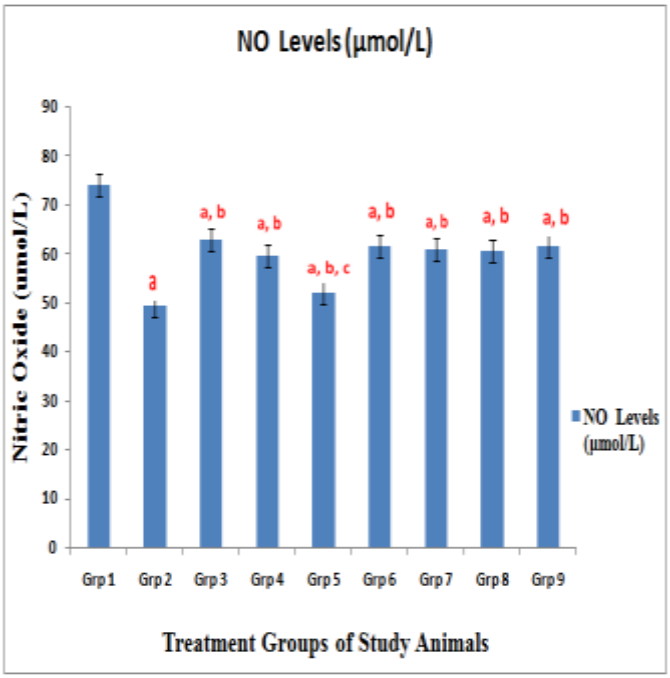

Figure I: Nitric oxide levels in methanolic extracts of *Curcuma longa* Rhizome and *Spondias mombin* leaf treated rats.

Note: MSM- Methanolic extract of *Spondias mombin* leaf; MCL- Methanolic extract of *Curcuma longa* rhizome; Pb- Lead acetate;

Grp=Group, Grp 1: Control (no treatment); **Grp 2**: Pb treated only; **Grp 3**: Pb+ vit.E treated; **Grp 4**: Pb+200mg/kg MSM treated; **Grp 5**: Pb+ 400mg/kg MSM treated; **Grp 6**: Pb + 500mg/kg MCL treated**;** **Grp 7**: Pb + 1000mg/kg MCL treated; **Grp 8**: Pb+200mg/kg MSM+ 500mg/kg MCL treated; **Grp 9**: Pb+400mg MSM+1000mg/kg MCL.

The effects of separate and co-administrations of methanolic extracts of *Curcuma longa* Rhizome and *Spondias mombin* leaf on serum Nitric Oxide (NO) level in the study rats followed thus: the mean values of all the groups except that of group 3 (Pb±100mg/kg Vit. E) (63.01 ± 2.05) significantly (P<0.05) decreased when compared to group 1 (Control) (74.30 ± 2.59). The mean nitric oxide levels in groups 3, 6 and 9 were significantly (P<0.05) higher when compared to group 2 (Pb treated only) (49.56 ± 1.33). The levels of nitric oxide in group 2 (Pb only) (49.56 ± 1.33) rats were significantly reduced when compared to group 3 (Pb + 100mg/kg Vit. E) (63.01 + 2.05). There was no significant difference (P>0.05) between group 8 (200mg/kg MSM + 500mg/kg MCL) (60.72 + 2.74) and 9 rats (400mg/kg MSM+1000mg/kg MCL) (61.55 + 2.37).

IV. DISCUSSION

Treatment of rats with lead acetate is known to deplete serum nitric oxide level in rats (Badavi *et al*., 2008). Significant depression in nitric oxide level may result in vasoconstriction leading to chronically elevated blood pressure−hypertension and other related diseases in the rats (Usman *et al.*, 2009).

From the results, treatment with high dose co-administered *Curcuma longa* rhizome and *Spondias mombin* leaf extracts 1000 and 400 mg/kg respectively was able to significantly elevate the depressed nitric oxide level in the lead treated rats than all other groups treated with the separate extracts. This result was in line with the findings of [14] and [19]. This is to say that high dose of these extracts possess the potential to restore depressed serum nitric oxide levels in the biological system.

V. CONCLUSION

From the results obtained in this study, it can be deduced that the separate doses of methanolic *Curcuma longa* rhizome and *Spondias mombin* leaf extract were able to ameliorate lead induced nitric oxide deficiency. However, the groups of rats co-administered with the methanolic extracts of *Curcuma longa* rhizome and *Spondias mombin* leaf (especially the high doses of 1000/400 mg/kg respectively) showed better ameliorative effect in the management of nitric oxide deficiency. In conclusion, the marked elevation of serum nitric oxide levels following the co-administration of the methanolic *curcuma longa* rhizome and *spondias mombin* leaves reveal their possible synergistic potentials in stabilizing nitric oxide deficiency.

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