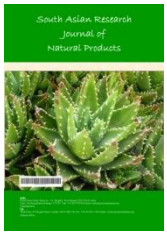

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In vivo Antioxidant Activity and Haematological Effect of Powdered Turmeric (*Curcuma longa* Linn.) Supplement in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present study was designed to evaluate the haematological effect and degree of *in vivo* antioxidant activity of powdered turmeric supplement using wistar rats as model. Fifteen male animals were randomly divided into 3 groups - Group A (Control) received 1ml of distilled water, Group B received 500 mg/kg turmeric and Group C received 1000 mg/kg turmeric by oral gavage daily for 28 days. On day 29, blood samples were collected for haematology and Antioxidant assay. Haematology was done by estimating the level of haematocrit, haemoglobin concentration, erythrocyte count, leucocyte count, platelet count and differential leucocyte count (Neutrophils, lymphocytes, Eosinophil, and monocytes). The antioxidant activities were estimated by assaying for the oxidative biomarkers such as Malondiadehyde (MDA), Catalase (CA), Superoxide Dismutase (SOD) and Glutathione (GSH). Turmeric had no significant ($P > 0.05$) effect on blood parameters as well as serum SOD, GSH and CA level relative to the control. However, turmeric caused a significant ($p<0.05$) reduction in serum MDA levels. It is therefore concluded that powdered turmeric supplement as used in this study, has *in vivo* antioxidant activity with no effect on blood parameters.

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Keywords: Blood; superoxide dismutase; catalase; malondialdehyde; glutathione.

1. INTRODUCTION

For several centuries, medicinal plants have been used in the management of diseases. In recent times, there appears to be a rise in the utilization of medicinal plants for a variety of therapeutic purposes. Natural products from some plants are used in pharmaceutical preparations either as pure compounds or as extracts [1] and their effectiveness is based on the various secondary metabolites they possess, which include amongst others, alkaloids, terpenes, glycosides, flavonoids, saponins. Amongst these medicinal plants with global use is *Curcuma longa.*

Curcuma longa, also known as turmeric, is a herbaceous perennial plant belonging to the ginger family, Zingiberaceae [2]. *Curcuma longa* has been used for years as a traditional remedy in the management of a wide range of diseases and has been reported to possess antioxidant, anti-inflammatory, antimicrobial, antitumor and hepatoprotective activities [3]. The phenolic turmeric pigment, curcumin, has been linked to this anti-inflammatory, anti-carcinogenic and antioxidant properties [4]. According to Durgaprasad et al. [3], curcumin is said to have potent antioxidant properties comparable to those of vitamins C and E. The roots are very vital and utilized as a religious, culinary, and medicinal agent. According to Miquel et al. [5], the powdered form of this rhizome is generally used as a food additive in form of spice to impart flavour and yellow colour to food.

Undoubtedly, turmeric has been purported to have antioxidant properties using turmeric extracts and curcumin isolate, but this study used powdered turmeric supplement, which is the traditional form used by the general public and traditional medicine practitioners, to determine the level of *in vivo* antioxidant activity associated with turmeric and its haematological effect in wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Processing

Dried rhizomes of turmeric (*Curcuma longa* Linn.) were purchased from Modern market, Makurdi Metropolis, Benue State, Nigeria. The Authentication was carried out at the Ecoland Herbarium, Port Harcourt, Nigeria. The specimen voucher number of the plant is EH / P/ 069.

A 3.3kg portion of the rhizomes was milled at Ozuoba Market, Obio-Akpor Local Government Area of Port Harcourt, Rivers State, to obtain a fine powder. The powder was further dried after milling to remove any residual moisture, before passing it through a fine sieve. The obtained turmeric powder was used for the study.

2.2 Acute Oral Toxicity Study

The acute oral toxicity study for the dried turmeric powder was carried out to determine the LD_{50} of the powder according to the method of Lorke [6], using a total of 18 male wistar rats. This aspect of the study was done not only to determine the LD_{50} of the powder, which is vital in identifying its clinical effects following oral administration, but also to determine the doses to be used in the study [7].

2.3 Experimental Animals

In this study, fifteen adult male wistar rats (weighing 135-175g) were purchased from the Pharmacology Department of the University of Port Harcourt. They were kept in Animal House for a week before the start of the experiment and all through the duration of the study, with free access to food (Top Feeds Nigeria Limited®) and tap water. All experimental animals were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the institution.

The animals were assigned into three (3) groups of five (5) rats each. The powdered turmeric supplement was reconstituted with distilled water to get a stock solution of 100mg/ml, which was administered to the animals for 28 days by oral gavage according the doses assigned to their groups as follows:

Group A (Control) received 1ml of distilled water (vehicle)

Group B received 500 mg/kg dose of powdered turmeric.

Group C received 1000 mg/kg dose of powdered turmeric.

At the end of the experiment, the animals were anaesthetized, and 5ml of blood sample was

collected from the retro-orbital plexus of each rat for the evaluation of the oxidative biomarkers and haematological parameters using plain sample bottles and EDTA bottles respectively.

(a) Estimation of Oxidative Biomarkers

The blood samples collected in plain bottles (4 mls of blood per rat) were allowed to stand for 30 - 45 mins to coagulate and then centrifuged for 15 mins at 3000 rev/min to obtain serum. The serum was then tipped into a separate vial, placed in microcentrifuge tubes, capped, and stored at -20°C until analysis. The serum was later subjected to oxidative stress markers estimation as follows: Malondialdehyde (MDA) was determined measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa, Ohishi and Yagi [8]. The method of Sedlak and Lindsay [9] was followed in estimating the level of reduced glutathione (GSH) level. Catalase (CA) activity was determined according to Clairborne [10]. The activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich [11].

(b) Haematological Parameters

The EDTA blood samples (1ml of blood per rat) were used for the estimation of haematological parameters such as packed cell volume (PCV), haemoglobin concentration (HB), erythrocyte count, leucocyte count (WBC), platelet count and differential leucocyte count which includes Neutrophils, eosinophils, lymphocytes and monocytes.

2.4 Statistical Analyses

All values were expressed as mean ± SEM. The data were statistically analyzed using one-way Analysis of Variance (ANOVA) and significant means were separated using the Tukey method. The level of statistical significance was set at 95% confidence interval (P >0.05).

3. RESULTS

3.1 Acute Oral Toxicity Testing

The acute oral toxicity testing of the powdered turmeric supplement revealed that the supplement was not toxic at the highest dose of 5000mg/kg used in Lorke's method. This was evidenced by the absence of mortality and other signs of toxicity in the treated rats. Based on that finding, $^{1}/_{10}$ and $^{1}/_{5}$ of the maximum dose (5000 mg/kg) was adopted as the doses for the study.

3.2 *In vivo* Antioxidant Activity

The treatment of Wistar rats with powdered turmeric supplement for 28 days resulted in a dose-dependent non-significant ($P<0.05$) increase in the mean serum level of Catalase relative to the control (Fig. 1). Fig. 2 shows that treatment of rats for 28 days with 500 and 1000 mg/kg doses of powdered turmeric supplement produced a highly significant ($p<0.01$) decrease in the mean serum malondialdehyde level relative to the control. Following a 28-day treatment period, powdered turmeric supplement did not produce any significant ($P>0.05$) effect in the mean serum level of glutathione (GSH) in comparison with the control (Fig. 3). Fig. 4 shows that powdered turmeric supplement did not produce any significant ($p<0.05$) change in the mean serum level of Superoxide Dismutase of treated rats in all the test groups relative to the control.

3.3 Haematological Effect

The haematological profile of experimental rats given 500 and 1000 mg/kg doses of powdered turmeric supplement is presented in Table 1. After the 28-day study period, no significant ($P>0.05$) change was recorded in the mean blood parameters of the test groups relative to the control (Table 1).

4. DISCUSSION

Natural products are tiny molecules that are produced naturally by a variety of sources including plants and animals. They exert both positive and negative effects by altering biological targets and pathways involved in oxidative stress and antioxidant response [12]. Damage to biological systems results from an imbalance between the generation of oxidants and antioxidant defenses, thus resulting in oxidative stress. Oxidative stress contributes to many pathological conditions and diseases as a result of its overwhelming impact on the antioxidant systems.

Endogenous antioxidants defense against the reactive oxygen species (ROS), the precursors of oxidative stress, are strengthened by natural antioxidants present in natural products. In traditional medicine, natural products and herbal formulations possessing antioxidant properties have been used to treat a variety of diseases [13]. Adwas et al. [14] stated that high consumption of natural foods that are rich in

antioxidants will provide more protection against toxic agents and related diseases. Natural antioxidants have a variety of biochemical actions such as inhibition of the production of ROS and scavenging of free radicals" [15,16].

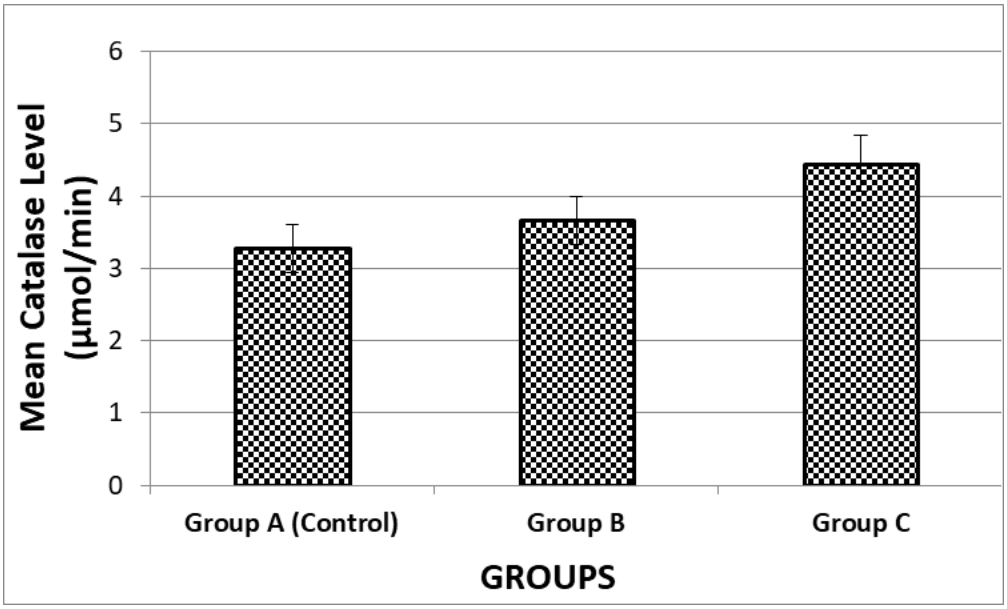

Fig. 1. Effect of powdered turmeric supplement on catalase level in wistar rats treated for 28 days

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at 95% confidence interval (P >0.05).
Groups A, B and C represent the control rats given 1ml of distilled water, 500 mg/kg treated rats and 1000 mg/kg treated rats, respectively

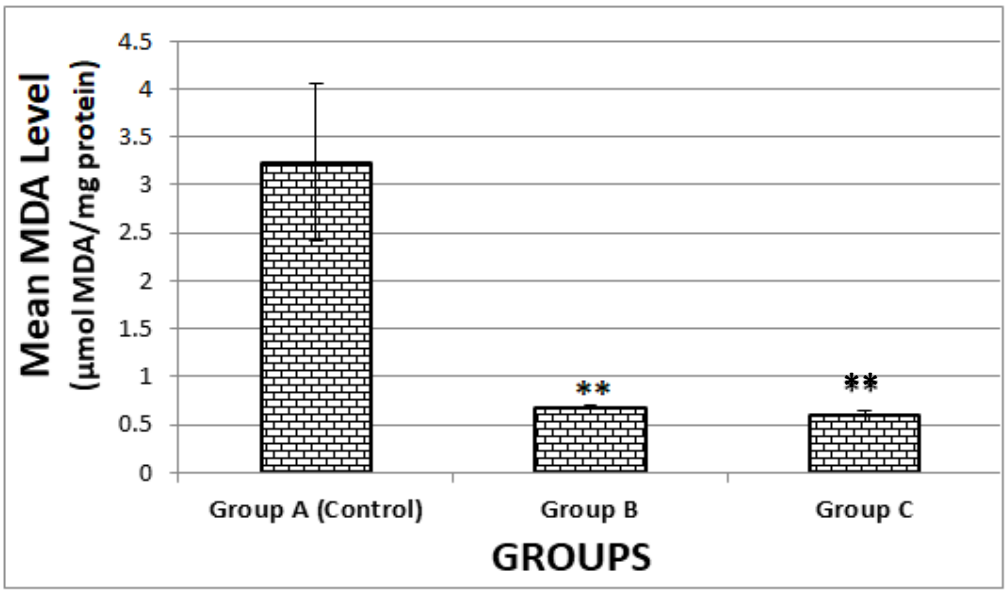

Fig. 2. Effect of powdered tumeric supplement on malondialdehyde (MDA) level in wistar rats treated for 28 days

*Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). *p<0.05, **p<0.01 vs. Control. Groups A, B and C represent the control rats given 1ml of distilled water, 500 mg/kg treated rats and 1000 mg/kg treated rats, respectively.*

Table 1. Effect of powdered supplement of turmeric on haematological parameters in wistar rats treated for 28 days

Parameters	GROUPS		
	Group A (Control)	Group B	Group C
Haematocrit (%)	33.75±2.10	33.40±0.87	35.25±0.85
Haemoglobin Conc. (g/dL)	16.13±0.87	15.72±0.34	16.90±0.53
Erythrocyte Count ($X10^{12}$/L)	5.95±0.41	5.96±0.11	6.23±0.14
Total Leucocyte Count ($X 10^9$/L)	15.45±0.76	18.16±2.79	18.00±4.90
Platelet Count ($X10^9$/L)	311.50±32.98	306.60±49.69	319.50±16.32
Neutrophils (%)	27.75±0.85	26.40±1.57	33.75±4.73
Lymphocytes (%)	64.25±1.03	64.40±1.83	56.50±5.04
Eosinophils (%)	2.50±0.29	2.80±0.37	3.00±0.41
Monocytes (%)	5.50±0.65	6.40±0.51	6.75±0.95

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference exists across the table at 95% confidence interval (p >0.05). Groups A, B and C represent the control (given 1ml of distilled water), 500 mg/kg treated rats and 1000 mg/kg treated rats, respectively

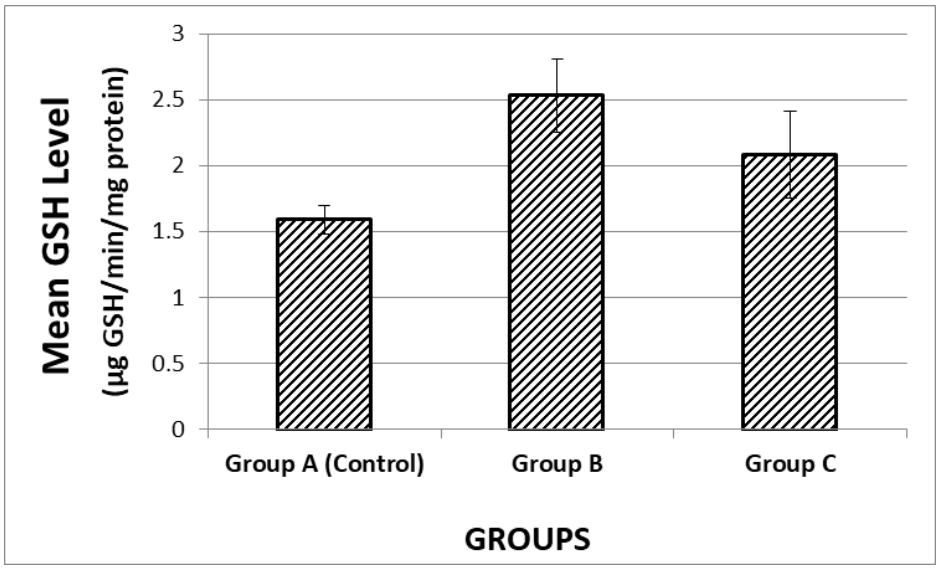

Fig. 3. Effect of powdered tumeric supplement on glutathione (GSH) level in wistar rats treated for 28 days

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at 95% confidence interval (P >0.05). Groups A, B and C represent the control rats given 1ml of distilled water, 500 mg/kg treated rats and 1000 mg/kg treated rats, respectively

From this study, *Curcuma longa* rhizome, which is a natural product from plant source, markedly decreased the serum level of Malondialdehyde (MDA), an oxidative stress marker, with no significant effect on the serum levels of endogenous antioxidants – Superoxide Dismutase (SOD), Catalase (CA) and glutathione (GSH).

MDA is one of the byproducts of polyunsaturated fatty acid peroxidation in cells [17]. A rise in free radicals - reactive oxygen species, leads to overproduction of MDA. Serum MDA levels could reflect the extent of lipid peroxidation as well as cell membrane and DNA damage [18]. Therefore, the decrease in MDA in this study shows that powdered *Curcuma longa* rhizome reduced the oxidative reaction in the body, and the rate of MDA accumulation by mopping up the free radicals that produced it, thus a proof of its *in vivo* antioxidant activity. In line with our finding, Jeber & Tawfeek, [19] reported that turmeric oil decreased the serum MDA level of adult male rats after a 60-day treatment, though with an increase in their GSH level.

SOD, GSH, and catalase are antioxidant enzymes that scavenge the free radicals and are components of the body's protective antioxidant networks [20]. Although there was a decline in

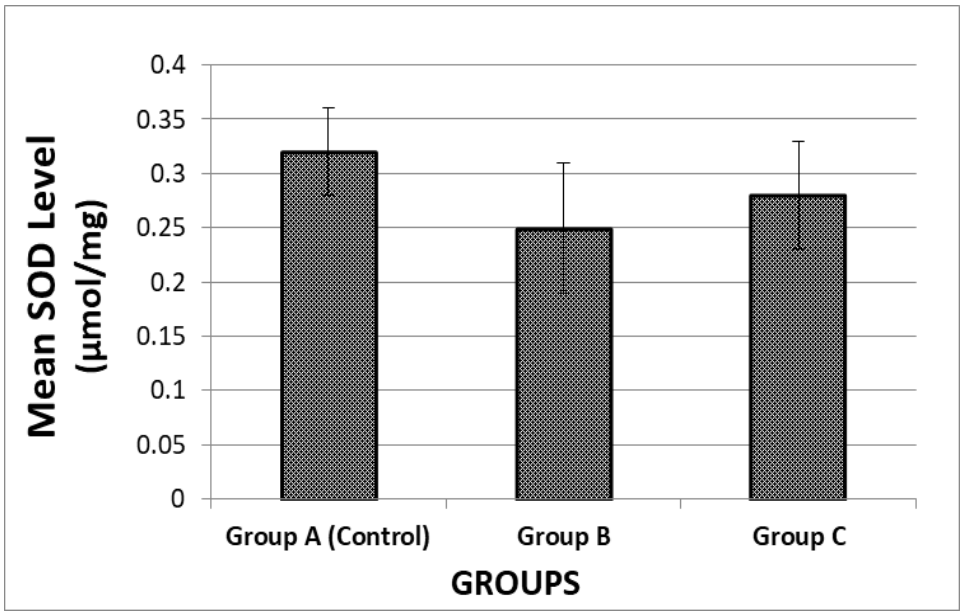

Fig. 4. Effect of powdered turmeric supplement on superoxide dismutase (SOD) level in wistar rats treated for 28 days

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at 95% confidence interval (P >0.05). Groups A, B and C represent the control rats given 1ml of distilled water, 500 mg/kg treated rats and 1000 mg/kg treated rats, respectively

serum MDA level by powdered *Curcuma long* rhizome, its effect on the antioxidant enzymes was not obvious. The reason for this could be in the yet-to-be-established mechanism of action of *Curcuma longa* rhizome which may be associated with its bioactive compounds. This result is in line with the work of Qasem et al. [21] who found that the total antioxidant capacity in tissues and serum of broiler chicken was not significantly affected by dietary supplement of turmeric powder (*Curcuma longa*) even at higher doses of 10, 12, 14, 16, 18 and 20 g/kg. However, Hosseini-Vashan et al. [22] reported that in heat-stressed broiler chicks given turmeric powder as a feed additive, the activity of antioxidant enzymes (glutathione peroxidase and SOD) increased. *Curcuma longa* rhizome powder contains curcumin, a potent antioxidant and anti-inflammatory agent [23,24], whose efficiency may be increased under stressful circumstances. In the present study, the experimental animals were not subjected to stressors which should elicit the activity of the endogenous antioxidant enzymes; this may be the basis for the disparity in the results.

Powdered *Curcuma longa* rhizome did not affect the blood parameters of male rats under the conditions of this experiment. This could be attributed to the health status of the animals which in the absence of stressors are seen to be apparently healthy.

5. CONCLUSION

Under the conditions of this experiment, it is concluded that powdered turmeric supplement as used in this study, has *in vivo* antioxidant activity with no effect on blood parameters.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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