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Possible Antioxidant and Haematinic Properties of the Stem Bark of *Theobroma cacao L.* in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: This study investigated the potential antioxidant effects of aqueous extract of the stem bark of *Theobroma cacao L.* in Wistar rats.
Methods: Twenty Wistar rats weighing between 126 g – 224 g were grouped randomly into 4groups of 5 rats each. Group 1 served as control and received water while groups 2, 3 and 4 rats were given 1000mg/kg, 3000mg/kg and 5000mg/kg b.wt of the extract respectively for 28days. On the 29th day, the rats were anaesthetized and blood samples were collected for analysis of some haematological parameters, enzymatic and non- enzymatic antioxidant activities.
Results: The results obtained showed that there was significant increase ($p<0.001$) in SOD, Catalase activities and MDA levels in a dose dependent manner. The results also showed significant increase ($p<0.001$) in RBC Group 2, 3 and 4 rats when compared to the Group1. Significant increase was also observed in Hemoglobin (Hb) and Hematocrit (Hct) level in group 2 and 3 rats ($p<0.001$). Mean corpuscular volume was significantly increased in group 2 rats

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(*p<0.001*).
Conclusion: The findings from this study showed the antioxidant and hematinic potentials of the stem bark of *Theobroma cacao L.*The aqueous extract of the stem bark of Theobroma cacao L. has a potential antioxidative and hematinic effects in Wistar rats. This is largely due to its rich phytochemical and nutritive contents. Further research work will be needed to see the possible application of these properties in humans.

Keywords: Theobroma cacao L.; antioxidant properties; haematological parameters; wistar rats.

1. INTRODUCTION

Herbs are available to humans and have been explored to the maximum for their medicinal properties. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. Various parts of plants like roots, leaves, bark exudates etc. are used as per medicinal properties to prevent, allay symptoms or revert abnormalities back to normal [1,2,3]. This is primarily because of the general belief that herbal drugs are without any side effects, besides being cheap and locally available [4].

Phytochemicals are a large group of plant-derived compounds hypothesised to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, and plant-based beverages such as Tea [5].

Antioxidants help to neutralize the oxygen-based free radicals that are present in the body. Natural antioxidants from herbs protect the living system from oxidative stress and other chronic diseases, and thereby contributes positively to health. [6,7,8].

The system is composed of compounds such as superoxide dismutase (SOD). This is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Catalase (CAT) reacts with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, phenol) using 1 mole of peroxide in a kind of peroxidase activity: glutathione peroxidase (GSH-Px), glutathione (GSH), glutathione reductase (GSSH). Glutathione peroxidase, catalyze the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GPX, thereby protecting mammalian cells against oxidative damage. Peroxidation of membrane lipids, most notably the polyunsaturated fatty acids arachidonic acid and linoleic acid, generates a wide array of molecules, such as lipid hydroperoxides, which are secondary lipid peroxidation products (for example, malondialdehyde and 4-hydroxynonenal, HNE). Lipid peroxidation products can damage membrane structure with the formation of membrane pores, alter water permeability, decrease cell deformability, and enhance IgG binding and complement activation [9]. Others include: Glucose-6-phosphate dehydrogenase (G6PD) [10], glutaredoxin and thioredoxin systems [11], uric acid, vitamins A, C, E as well as protein and DNA repair enzymes [12,13,14].

A comparative research study has made it evident that *Theobroma cacao* (*T.cacao*) exhibits higher antioxidant activity than black tea, green tea and red wine [15,16]. The phenolic compounds and other constituents such as flavonoids, tannins, triterpenes, and saponins contained within the plants are also determined to have antioxidative activities [17,16]. Long term feeding studies of cocoa (cocoa powder) showed an increase in total plasma antioxidant capacity and a reduction in susceptibility to oxidative injuries [18]. Indeed *Theobroma cacao* products such as seeds, leaves etc. have been widely used in the field of herbal medicine for diverse diseases. The stem back is readily available and may not infringe on the yield and economic impact of the plant. There is dare data scarcity of the stem bark of *Theobroma cacao.* Our earlier work (under peer review) revealed higher concentration of phytochemicals present in the cocoa seeds and other products in the stem bark.

The study was designed to investigate the possible antioxidant and haematinic properties of the aqueous extract of the stem bark of *Theobroma cacao L.*

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh stem barks of *Theobroma cacao* were collected from Adedeji farm, at Igbajo, Boluwaduro L.G.A of Osun State. They were authenticated and a sample of the plant parts deposited at the herbarium of the Department of Plant Science and Biotechnology of University of Portharcourt (Ref. No. UPH/PSB/2017/038).

The fresh stem barks of *Theobroma cacao L.* were air dried for 10 days to remove sufficient moisture. The dried sample was cut into smaller pieces and further pulverized with the aid of manual mill. The powdered plant sample was stored in an air tight container for further analysis. 500g of the pulverized stem barks was macerated in 2400ml of distilled water in warm water bath at 45^0c for 48 hours and then filtered with cheese cloth. The marc was rewashed until all the extractable constituents were completely washed out and then filtered. Both filtrates were combined, filtered with Whatman (No1) filter paper and concentrated at 45^0c. Percentage yield was 2.5. The extract was stored in the fridge until when needed.

2.2 Experimental Design

Twenty female Wistar rats, were used for this study. The rats were ages 10-12 weeks and weighed between 126-224 g. They were placed in separate cages in the animal house of the Department of Physiology, Faculty of Basic Medical Science Madonna University, Elele, Rivers State of Nigeria under natural day and night cycles. They had free access to normal rat pellets and watered ad libitum. They were allowed two weeks of acclimatization to their environment. The cages were cleaned daily to prevent infection of the animals; care and treatment were conducted in conformity with the institutional guidelines which are in compliance with the guide for the care of laboratory animals, United States National Research Council, 1996. The rats were grouped randomly into 4 groups of 5 rats each.

Group 1 served as control, received distilled water which was the vehicle.

Group 2 received 1000mg/kg, b.wt of the extract.

Group 3 received 3000mg/kg, b.wt of the extract.

Group 4 received 5000mg/kg b.wt of the extract.

All administrations were done for 28days.

The rats were sacrificed 24 hours after the last administration.

Blood sample was collected under anesthesia through cardiac puncture using a sterile needle and syringe. 2ml of blood was dispensed into EDTA bottles for Haematology analysis. Another 2ml were put into clean test tubes and was kept at room temperature for 30minutes to clot. Afterwards the test tubes containing the clotted blood samples were centrifuged at 3,000 revolutions per minute for 10 minutes using a table centrifuge to enable complete separation of the serum from the clot blood. The clear serum supernatant was carefully aspirated into clean dry sample bottle and stored in a refrigerator for biochemical analysis.

2.3 Biochemical Analysis

Using standard procedures [19], the oxidative stress biomarkers assayed for include the Antioxidant enzymes-Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) other oxidative stress biomarkers that serve as secondary products of lipid metabolism which include Malondialdehyde (MDA). SOD was assayed for, using iodophenol nitrophenol phenyl tetrazolium (INT), CAT was assayed for, using hydrogen peroxide. GPx analysis was done by measuring the rate of NADPH oxidation. MDA was assayed for, using Thiobarbituric acid.

2.4 Haematological Measurements

Blood samples were collected through cardiac puncture into sample bottles tubes coated with ethylene diaminetetra-acetic acid (EDTA). The samples were immediately analyzed for haematological parameters using automated Sysmex apparatus of the type 8999. The analyses were done according to the manufacturer's instructions. The parameters included: White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin (Hb), Haematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Platelets Count (PLT).

2.5 Statistical Analysis

Results were expressed as Mean ± SEM. All data obtained were analyzed using Analysis of Variance (ANOVA), followed by a post – hoc test of Fisher's Least Significant Difference for pair wise comparison. (SPSS 16 .0 for Windows). P values <0.05 were considered statistically significant.

3. RESULTS

Figs. 1 - 4 show the mean ± SEM of enzymatic and non-enzymatic antioxidant activity present in varied doses of aqueous extract of the stem bark of *Theobroma cacao L*.

A significant increase ($p<0.001$) in Superoxide dismutase (SOD) activity in group the group given 1000mg/kg b.wt.) (Group 2) when compared with the control. (Fig. 1).

There was a significant increase in Catalase activity ($p<0.001$) in groups given 1000mg, 3000 mg/kg & 5000 mg/kg b.wt (2, 3 and 4) respectively in a dose dependent manner when compared to the control.

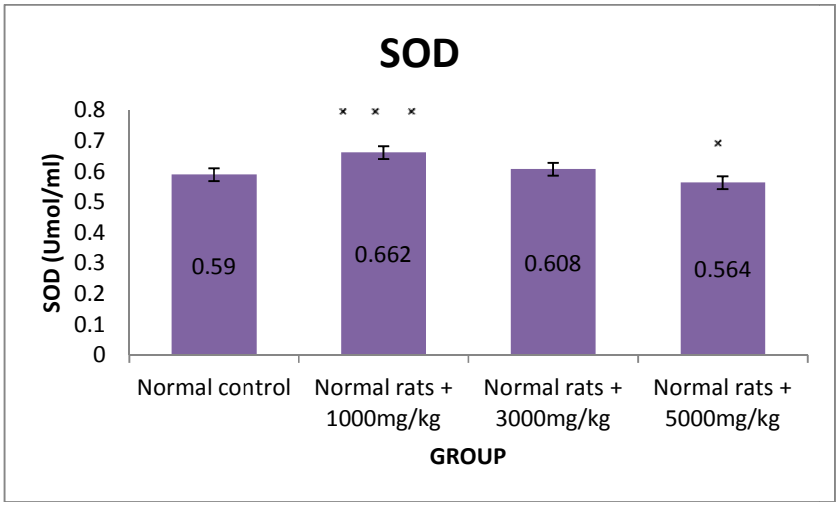

Fig. 1. Effect of *Theobroma L*. extract on Superoxide dismutase activity
Values are expressed as mean ± S.E.M. (n=5
****p<0.001 vs. control, *p<0.05 vs. control,*

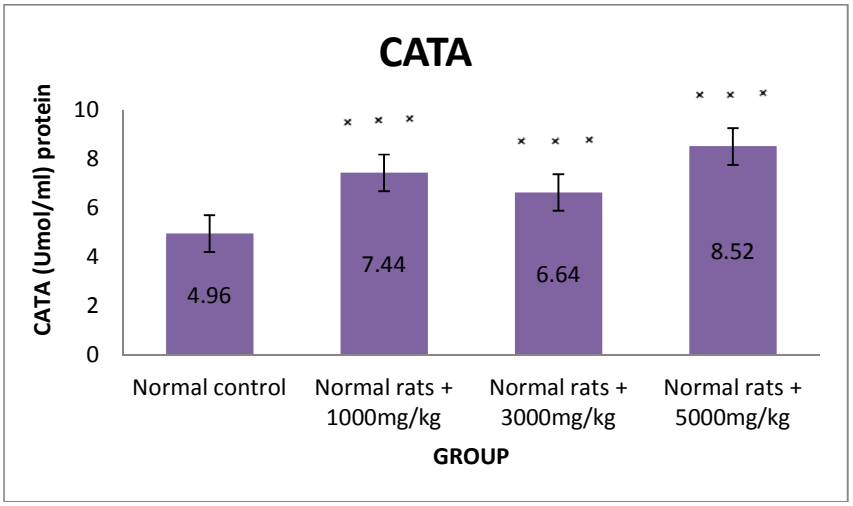

Fig. 2. Effect of *Theobroma L*. extract on catalase activity
Values are expressed as mean ± S.E.M (n=5)
****p<0.001 vs. control*

Glutathione activity showed no significant difference in all the groups when compared with the control. (Fig. 3).

A significant increase ($p < 0.001$) was observed in Malonyldialdehyde activity in all the treated rat groups when compared with the control (Fig. 4).

Table 1 represents the results of the haematological parameters investigated. There was significant increase in WBC and RBC counts, Haemoglobin and Haematocrit also showed significant increase in the groups 2 and 3, rats treated with 1000 and 3000mg/kg b.wt of the extract respectively, when compared with the normal control.

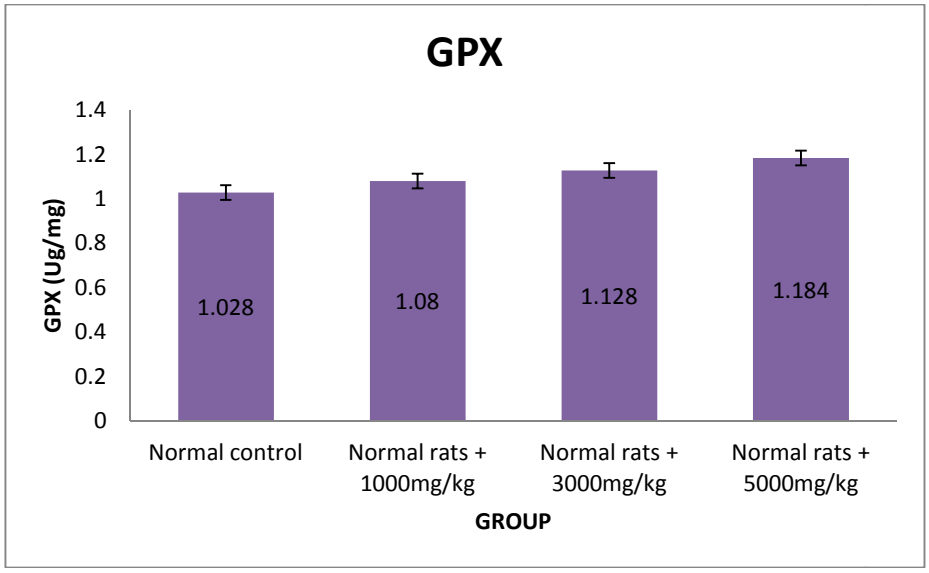

Fig. 3. Effect of extract on glutathione peroxidase activity
Values are expressed as mean ± S.E.M (n=5)
Significant at $p < 0.05$ vs. control

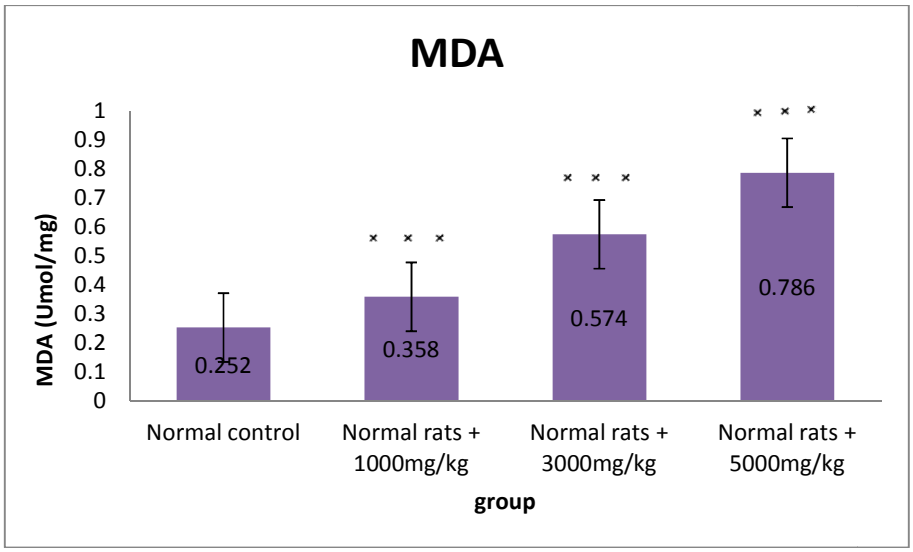

Fig. 4. Effect of extract on Malonyldialdehyde activity
Values are expressed as mean ± S.E.M (n=5)
****p<0.001 vs. control*

Table 1. Effect of aqueous extract of stem bark of *Theobroma cacao L*. on White Blood Cell Counts (WBC), Red Blood Cell Counts (RBC), Hemoglobin Concentration (Hb), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Platelet Counts in Wistar rats

Parameters Groups	Group 1	Group 2	Group 3	Group 4
White Blood Cell Count (WBC) (10^9/L)	4.05±0.06	5.29±0.11	5.75±0.01	7.83±0.06
Red Blood Cell Count (RBC) (10^12/L)	4.53±.02	4.73±.13*	5.09±.10***	4.97±.08***
Hemoglobin concentration (Hb) (g/ dL)	9.14±.04	9.92±.09***	10.02±.09***	9.26±.22
Hematocrit (HCT) (%)	27.98±.06	30.06±.49***	31.01±.25***	28.70±.13
Mean Cell Volume (MCV) (FL)	58.30±.89	65.74±.15***	60.80±1.0	57.92±.75
Mean Cell Haemoglobin (MCH) (PG)	19.06±.10	20.08±.13	19.60±.19	1.08±.22
Mean Cell Haemoglobin Concentration (MCHC) (g/dL)	32.67±.29	31.64±.26	32.30±.05	31.88±.17
Platelets Count (10^9/L)	164.80±1.28	128.00±0.45	125.00±0.55	262.60±0.68

Results are expressed as mean ± SEM (n=5), P is considered statistically significant at
**p<0.05 vs. control*
****p<0.001vs. Control*

4. DISCUSSION

Several studies have reported predominantly high antioxidant activity of cocoa and that its supplementation intervention duration of less than 1 week to as much as 12 weeks produce positive outcomes of its antioxidant ability. [18,20,21,22, which was also observed in the present study.

In this study, administration of aqueous extract of the *stem bark* of *Theobroma cacao L.* caused significant increase in Superoxide dismutase, Catalase and Gluthathione peroxidase (GPX) activities in the rats.

The significant increase in SOD and Catalase antioxidant activities in this study which verifies the antioxidant capabilities of the stem bark of cocoa were in agreement with the work done in rat models by Abrokwah [23].

The significant increase in all this oxidative stress biomarkers may be from the antioxidant properties of the polyphenols in cocoa, these antioxidants in the form of dietary polyphenols have the ability to modulate cellular sensor(s) for oxidative stress and thereby increase nitric oxide NO bioavailability [24,25]. The activities of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX) constitute a first line antioxidant defence system which plays a key and fundamental role in the total defense mechanisms and strategies in biological systems [26].

This antioxidant property of the stem bark may also be due to the presence of flavonoids such as epicatechin and catechin, proanthocyanidins, methylxanthines which have known antioxidant properties in addition to the innate physiologic reactive oxygen and nitrogen species (RONS) defense system [20,27].

Research has shown that alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species [28]. Previous phytochemical screening of *T. cacao (under peerreview)* revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins [29].

The results also revealed increased activity of Malonyldialdehyde in all the groups of normal rats administered with aqueous extract of *Theobroma cacao stem bark* progressively as the concentration of the extract increases. It appears that higher concentration of the extract may be associated with increased lipid peroxidation in the rats. Increased levels of TBARS (MDA) also indicate the excessive formation of free radicals and activation of lipid peroxidation system [30].

The outcome of this current study further confirms the haemato-stimulatory potentials of the extracts and therefore can serve as haematinics. No doubt, good and effective haematopoietic function can be enhanced and maintained by the use of micronutrients found in plants [31,32].

The extract however increasingly affect the hematological parameters of the rats in a dose dependent manner. The mechanism of action of the extract in building up red blood cell (RBC) may be attributed to its profile of important trace elements, proteins, vitamin (A, B and C), beta-carotene, amino acids, phenolic and iron [33].

Vitamin C increases iron absorption in the animal's body [34]. Vitamin A is necessary for many functions in the ruminants including vision, bone growth, immunity and maintenance of epithelial tissue and maintains adequate levels of iron in plasma that supply the different body tissues including the bone marrow [35].

Iron, which is commonly deficient in many plant-based diets, was found in abundance in this plant's leaves. Iron is a necessary component of haemoglobin and myoglobin for oxygen transport and cellular processes of growth and division [36].

The increase in platelet count could be as a result of the Vitamin K which has been shown to effectively increase platelet number [37,38], or vitamin C which is widely believed to also play a part in platelet number but without conclusive evidence.

5. CONCLUSION

In conclusion, the aqueous extract of the stem bark of *Theobroma cacao L.* has a potential antioxidative and hematinic effects in Wistar rats. This may be largely due to its rich phytochemical and nutritive contents.

DISCLAIMER

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical committee of The Faculty of Basic Medical Sciences, Madonna University, Elele. Rivers State. Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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