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Table of Contents: Volume 11 Number 6 June 2017

ARTICLE

Effects of ethanol and aqueous leaf extracts of Pterocarpus mildbraedii on hematology parameters and cholesterol in albino rats **28**
Adegbite A. Victor and Ezekwesili C. Nonyelum

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Full Length Research Paper

Effects of ethanol and aqueous leaf extracts of *Pterocarpus mildbraedii* on hematology parameters and cholesterol in albino rats

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One of the commonly consumed leafy vegetables in Nigeria is *Pterocarpus mildbraedii* Harms (Leguminosae) leaf. It has been found that, ethanol and aqueous extracts of the plant leaves exert anti-diabetic effect on rats. This study investigated the effects of ethanol and aqueous leaf extracts of this plant on some hematological parameters and serum cholesterol level in Wistar albino rats. Intraperitoneal administration of ethanol and aqueous extracts of *P. mildbraedii* leaves at the doses of 200 and 400 mg kg^{-1}, on two groups of rats for 28 days showed no adverse effects on the hematological parameters considered in this study. There was a significant ($p < 0.05$) dose dependent increase in almost all the hematological parameters. However, the serum cholesterol level was significantly reduced in rats exposed to both doses of the ethanol and aqueous extracts of the plant leaf as compared to the rats in the control group. These effects of the extracts increased with increased doses. These results suggest non-toxic effects of the leaf extracts of *P. mildbraedii* on the hematological parameters, as well as an ability to help in preventing cardiovascular diseases in the diabetics.

Key words: *Pterocarpus mildbraedii*, hematology, cholesterol, heart, packed cell volume, hemoglobin, toxicology.

INTRODUCTION

There are wide varieties of leafy vegetables consumed in Nigeria. Plant-derived foods, particularly vegetables and fruits, are beneficial components of the human diet. They contribute to life by providing different type of nutrients, vitamins and other substances (Gayatri et al., 2014) (Newman et al., 2003). The inclusion of a particular vegetable in the diet depends on a number of factors which include availability, indigenous knowledge and cultural practice (Jennifer et al., 2012; Eyo and Abel, 1983). Different authors have reported on chemical composition of the commonly used Nigeria leafy vegetables.

Nevertheless, a careful examination of literature

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revealed that, there are some less commonly used and inexpensive leafy vegetables whose nutritional potentials as well as their negative effects on living organisms have not been adequately studied. One of such is *Pterocarpus mildbraedii* Harms (Leguminosae) leaf which is locally known as Ora in Iboland (Adegbite et al., 2015; Akpayung et al., 1995), Nigeria.

P. *mildbraedii* grows mostly in the Eastern part of Nigeria. The young and tender leaves of this plant are used traditionally as vegetable for the preparation of soups and there has been claim that it possesses anti-diabetic properties.

Intake of vegetables, medicinal plants or drugs could cause alteration of the normal hematological and biochemical parameters of the body (Ayman, 2013). These alterations may therefore serve as indicators of adverse effects of these substances to the system.

Hence, evaluation of toxic properties of a substance is crucial when considering a substance for public health consumption. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects (Subramanion et al., 2011; Asante-Duah, 2002).

This study scientifically investigated the possible toxicological effects of ethanol and aqueous extracts of *P*. *mildbraedii* on some hematology parameters and cholesterol in albino rats.

MATERIALS AND METHODS

The fresh leaves of *P. mildbraedii* were collected from Abagana, Njikoka local Government area, Anambra State, Nigeria. The leaves were identified and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher specimen (NAU H. No. 162) was prepared and deposited in the herbarium. The leaves were separated from the stalk, air-dried at room temperature and ground into powdered.

Experimental animals

Forty (40) adult male Wistar albino rats weighing 200 ± 20.35 g were purchased from the animal house of the Veterinary Medicine Department, University of Nigeria, Nsuka. They were acclimated for two weeks in stainless metabolic rat cages in the animal house of Applied Biochemistry Department, Nnamdi Azikiwe University, Awka.

Chemicals

All the chemicals used were of analytical grade and were products of BDH Ltd, Poole, England.

Preparation of crude extracts

The extraction was carried out at room temperature with 500 g of the powdered leaves macerated in 2 L of water (distilled) for 72 h to obtain aqueous extract, while another 500 g of the powdered leaves in 2 L of 80% ethanol was used to prepare ethanol extract. The ethanol extraction was carried out with the use of soxhlet apparatus. The extracts were filtered through clean muslin cloth and the extraction process was repeated by adding another 2 L of distilled water and ethanol to the sample residue. The filtrate from each extraction was combined and the solvents evaporated with the aid of a rotary evaporator to obtain thick slurry of both extracts.

Study design

The animals were fed growers animal pellets from Guinea Feeds Nigeria PLC and water *ad libitum* for the period of the experiment (28 days). The animals were sorted and divided into five groups (A-E) according to their weights, replicated twice with each replicate having four rats. Group A served as the control which was not given the extracts of *P. mildbraedii* but were given 1 ml kg^{-1} of normal saline, groups B and C received 200 mg kg^{-1} and 400 mg kg^{-1} of ethanol extract of *P. mildbraedii* intraperitoneally, respectively while groups D and E were given 200 and 400 mg kg^{-1} of aqueous extracts of *P. mildbraedii*, respectively**.**

Studies on hematology parameters and serum total cholesterol

Effects of extracts on some hematology parameters

The rats were sacrificed on days 0, 14 and 28 of the administration. Three animals were sacrificed from each group on days 0, 14 and 28. The blood samples were collected into ethylenediaminetetra-acetic acid (EDTA) containing bottles to examine the possible effects of the extracts on certain hematology parameters such as packed cell volume (PCV), hemoglobin (Hb), platelets (PLT) and white blood cell (WBC).

Effects of extracts on serum total cholesterol level

The control animals (group A) were given only the normal feed and 1 ml kg^{-1} of normal saline. Two doses, 200 and 400 mg kg^{-1} body weight, of ethanol extracts of the plant leaves were administered intraperitoneally to groups B and C for 28 days. Two doses of aqueous extracts of the plant leaves were administered intraperitoneally to groups D and E for 28 days. The rats were sacrificed at days 0, 14 and 28 of the administration and the blood samples were collected into plain bottles to assay for serum cholesterol levels. The effects of the extracts on serum cholesterol level were examined. The cholesterol estimation carried out at day 0 before extracts administration was used as the baseline analysis, while those of days 14 and 28 were used as follow up analyses after the exposure of the rats to the extracts. The blood sample was collected into a non-anticoagulant containing bottle for serum cholesterol level analysis.

Statistical analysis

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS). Data were reported as mean ± SEM of 3 measurements and was analysed using ‘t’ test. *P* values less than 5% was considered significant ($p < 0.05$).

RESULTS

Effects of extracts on hematological parameters

Figures 1 and 2 shows the effects of ethanol and aqueous extracts

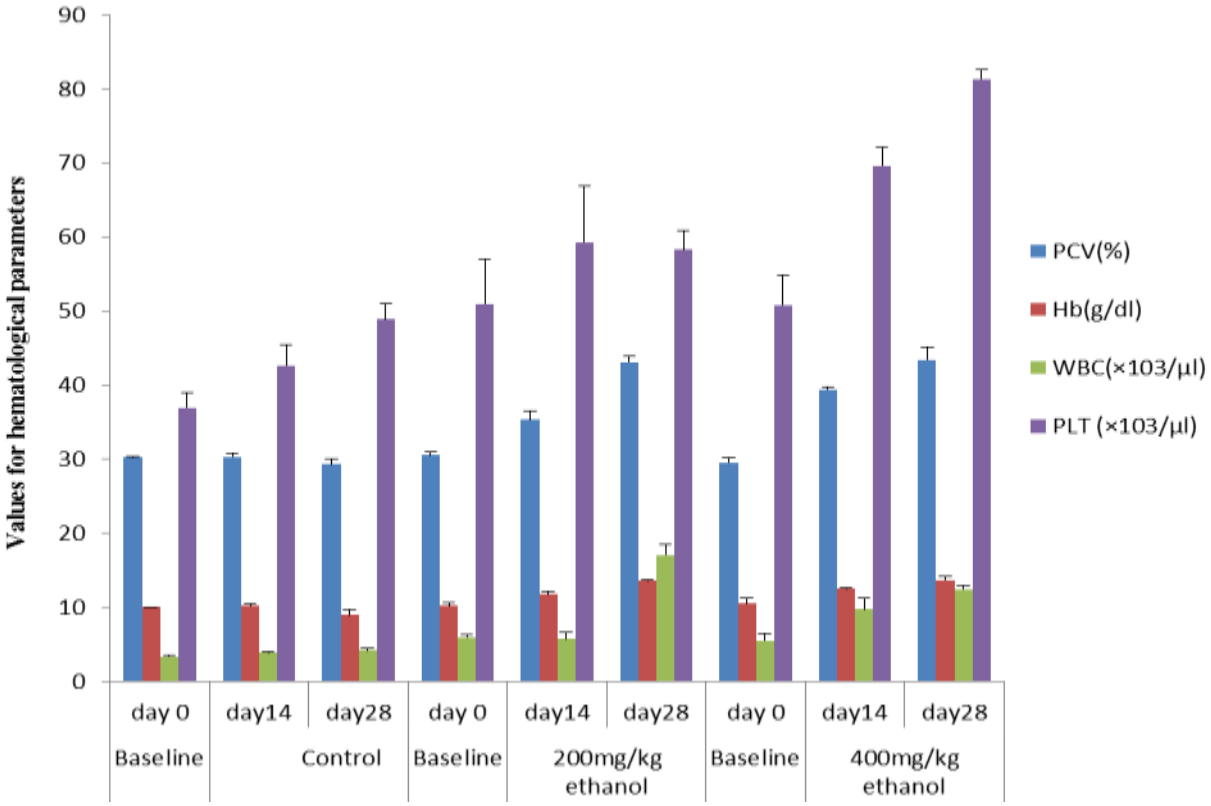

Figure 1. The effects of ethanol extract of *P. mildbraedii* on some hematology parameters.

of P. mildbraedii on some hematological indices of albino rats. The extracts of *P. mildbraedii* significantly ($p<0.05$) increased pack cell volume (PCV), hemoglobin (HB), white blood cell (WBC) count when compared with the results of baseline hematology tests carried out on all groups before treatments. Also, there were significant ($p<0.05$) increment in PCV, Hb, WBC and PLT values obtained from follow up hematology analysis when compared with the results of follow hematology analysis carried out on rats in the control group.

Effects of extracts on serum level of total cholesterol

Figures 3 and 4 shows the effects of ethanol and aqueous extracts of *P. mildbraedii* on serum total cholesterol level of albino rats. Both 200 and 400 mg kg^{-1} of ethanol and aqueous extracts of *P. mildbraedii* significantly ($P < 0.05$) reduced the serum level of total cholesterol in extracts treated rats when compared with the results obtained from baseline serum total cholesterol level. The same observation was obtained when the results obtained from serum total cholesterol analysis carried out on extracts treated rats were compared with those in the control group.

DISCUSSION

Phytotherapeutic products from medicinal plants are rapidly becoming popular in primary healthcare universally, with the peculiarity being the developing countries, and some have been regarded mistakenly as safe just because they are a natural source. Notwithstanding, these bioactive products from medicinal plants are presumed to be safe without any compromising health, and are thus widely used as self-medication (Vaghasiya et al., 2011).

Hence, toxicity study is needed vitally not only to identify the range of doses that could be harmful to human health but also to unveil the possible clinical signs caused by the substances under investigation (Ezekwesili et al., 2016; Rang et al., 2001). The results obtained from animal studies will be crucial in giving a definitive judgment concerning the safety of the plants if they are discovered to have sufficient nutritional and therapeutic potentials for development into pharmacological products (Marco et al., 2013; Moshi, 2007).

The 24 h acute toxicity study of the intraperitoneally administered ethanol and aqueous extracts of *P. mildbraedii* leaves revealed that their median lethal doses (LD_{50}) were 1258 and 1778 mg kg^{-1} body weights,

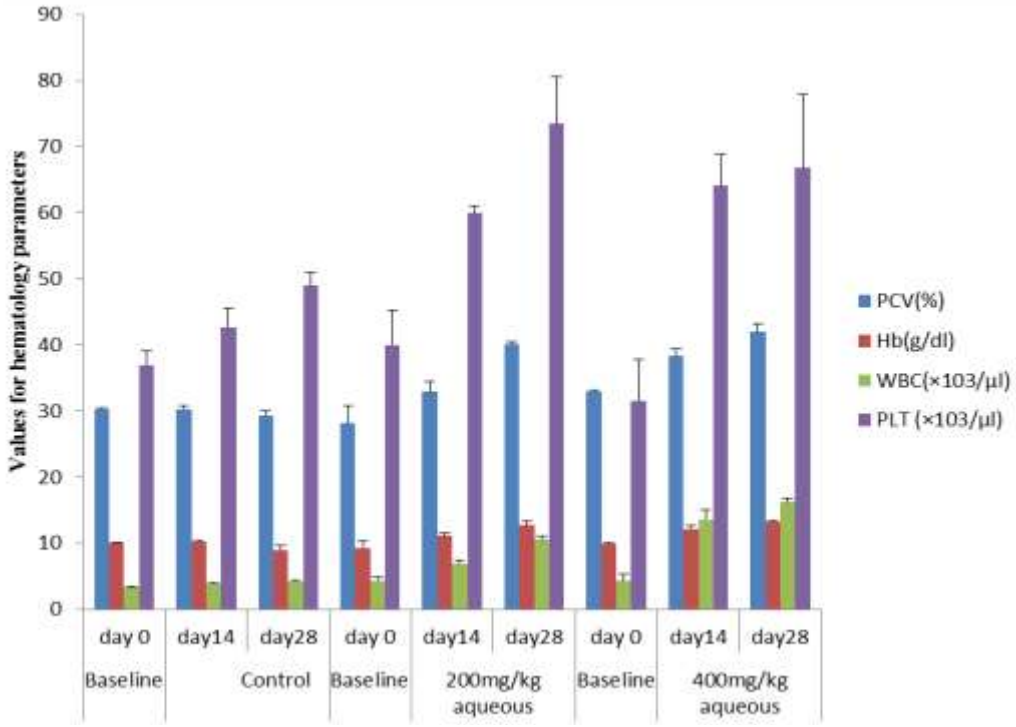

Figure 2. The effects of aqueous extract of *P. mildbraedii* on some hematology parameters.

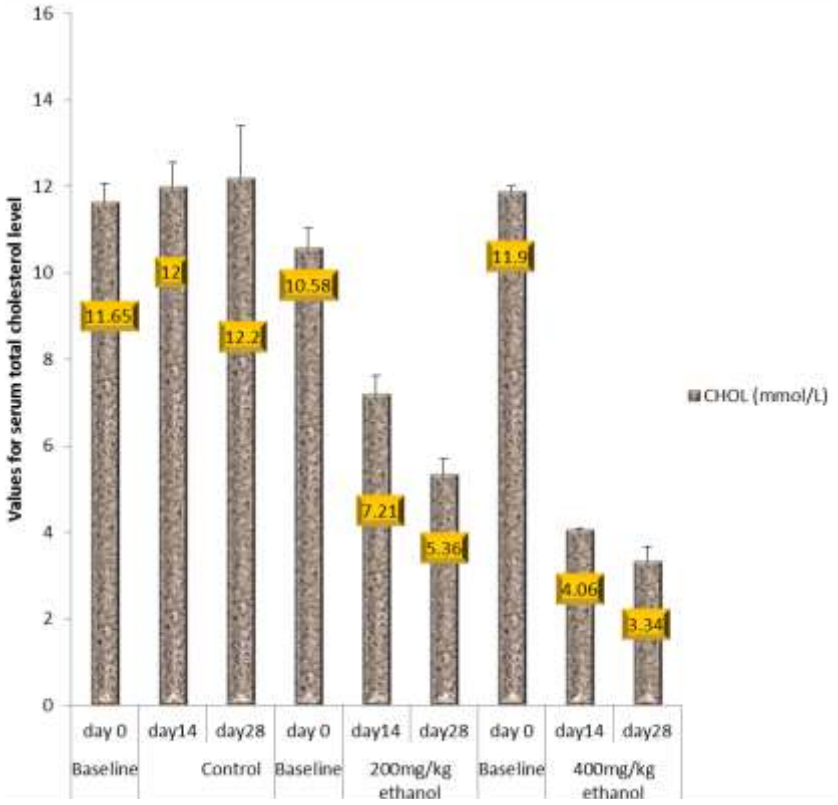

Figure 3. The effect of ethanol extract of *P. mildbraedii* on serum total cholesterol.

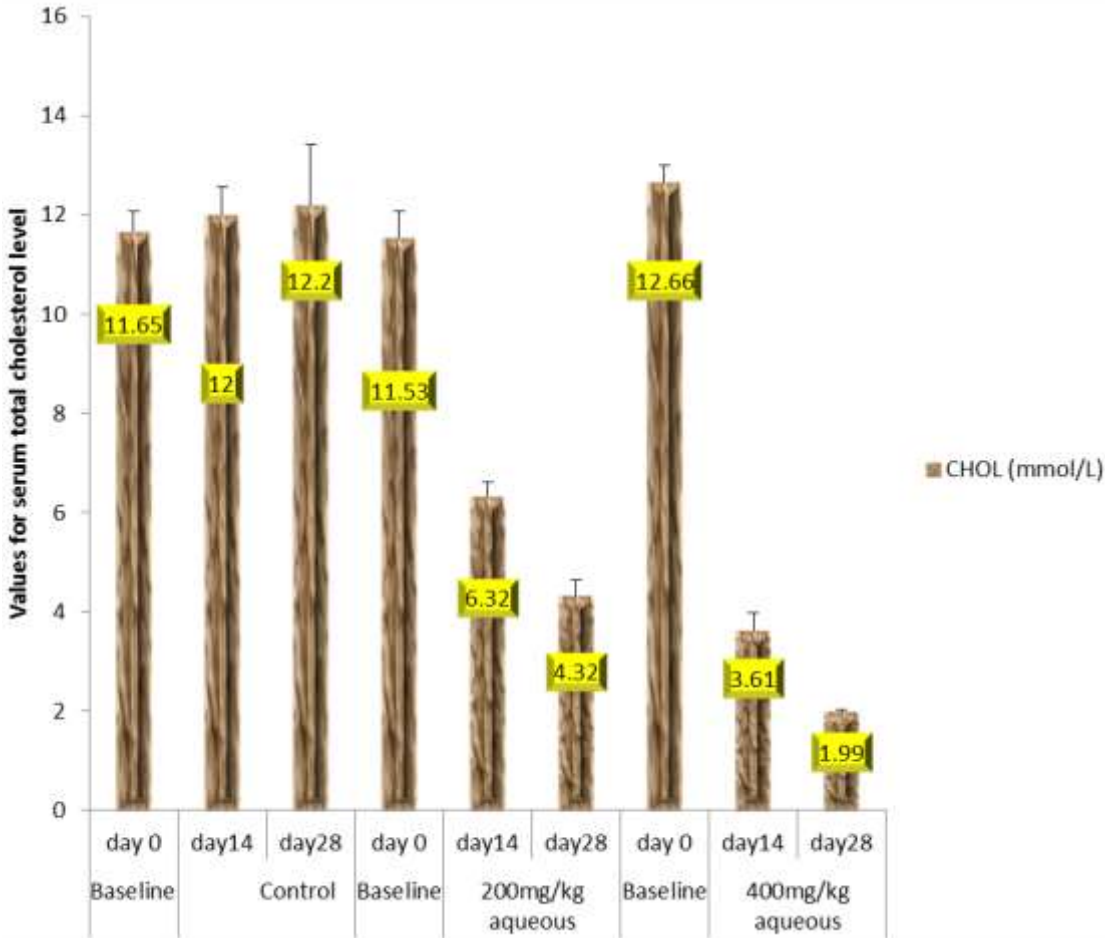

Figure 4. The effect of aqueous extract of *P. mildbraedii* on serum total cholesterol.

respectively. Finding from the study revealed that both doses of the ethanol and aqueous extracts of *P. mildbraedii* leaves did not have adverse effects on the hematology parameters considered in this study but boost the blood levels of some parameters.

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status in both animals and humans (Kulkarni and Veeranjaneyulu, 2012; Adeneye et al., 2006). There was a significant ($p< 0.05$) increase in almost all the hematological parameters considered in this study when comparing the results obtained from rats in control group with the extracts treated rats. However, the increment was also found to vary with the dose and the duration of exposure of rats to the extracts as shown in Figures 1 and 2. The 200 mg/kg ethanol extract of *P. mildbraedii* significantly increased WBC, PLT and RBC but PCV and Hb were not significantly ($p > 0.05$) increased in extract treated rats. However, all the hematological parameters considered were significantly ($p < 0.05$) increased by day 28 of exposure, except RBC.

The increaset in the hematology parameters was significant in extract treated rats when compared with the results obtained from baseline hematology analysis carried out on all rats before exposure and the control groups. All the hematology parameters considered in this study were significantly increased at 400 mg/kg of both ethanol and aqueous extracts of the plant leaves. Goh et al. (2016) observed a relationship between some metabolic diseases and leukocytes. Diabetes mellitus is also commonly associated with reduced hemoglobin concentration (Eunjin and Chulwoo, 2012; Thomas et al., 2003). The moderate WBC boosting effect of the extract can help in the protection against microbial infection. The red blood cells and hemoglobin level boosting effects could therefore be beneficial to the diabetic as well as anemic patients.

All the doses of ethanol and aqueous extracts of the plant considered in this study significantly ($p > 0.05$) reduced the serum cholesterol level in extracts treated rats when compared with the results obtained from rats in the control group. These results showed that the extracts

can be of help in preventing cardiovascular diseases associated with excess cholesterol level in the blood. These observations are in agreement with earlier report (Ezekwesili et al., 2016; Mohire et al., 2007) that at a higher dilution, the aqueous extracts of *P. marsupium* possesses an excellent cardiotonic activity. The effects of 200 and 400 mg/kg of aqueous extract of the plant on the total cholesterol were more ($p > 0.05$) significant as compared to the effects of the same doses of ethanol extract of the plant. This showed that, the aqueous extract of the plant was more potent in lowering the serum cholesterol level when compared with the ethanol extract.

Conclusions

The present results revealed that the leaf extracts of *P. mildbraedii* do not cause any apparent *in vivo* adverse effect on the hematopoietic system and also reduced the serum cholesterol level when studied in rat model. No death or signs of toxicity were observed in rats treated with both ethanol and aqueous extracts at doses of 200 and 400 mg/kg; thus, establishing safety in the use of *P. mildbraedii* leaf extracts.

Hence, the use of *P. mildbraedii* can be encouraged both as a leafy vegetable and as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost. A detailed experimental analysis of its chronic toxicity is essential.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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