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Comparative effect of ethanol extract of *Moringa oleifera* leaf and Boo-Sta Plus on the body weight and haematological indices of Wister albino rats

Ada Ak. Akwari*, Etah E. Nkanu and Elvis M. Ayim

Department of Animal and Environmental Biology, Faculty of Biological Sciences, Cross River University of Technology, Calabar, Nigeria.

*Corresponding author. Email: perfectgrace74@gmail.com; Tel: +234(0)8062839987/+234(0)8036080310.

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ABSTRACT: The comparative effects of ethanol extract of Moringa *oleifera* and Boo-Sta Plus on the red blood cell indices and body weight of Wistar albino rats were investigated. Fortification of food is not just trending in the human diet but also in the animal diet. Boo-Sta Plus have been employed in dietary fortification to achieve better results in meat quality, protein mass and weight gain, however, these artificial fortification causes antibiotic resistance and may have other negative health implications. Focus is now being directed on plant-based alternatives such as Moringa *oliefera* to curb these negative effects. Fresh leaves of Moringa *oleifera* were washed, oven dried and ground. Twenty (20) Wistar rats weighing (150-170 g) were divided into four groups containing five rats in each group (A, B, C and D). Rats in group A (control) received normal rat chow feed and water. Group B was fed 200 mg/kg body weight Moringa extract (low dose), Group C received 400 mg/kg body weight (high dose) and Group D received Boo Sta-Plus. All the experimental groups received their respective treatment in addition to feeding and water. Weekly body weight was recorded during the duration of 21 days of administration of leaf extract. At the end of 21 days of treatment, blood samples were obtained through cardiac puncture and analysed for haematological indices. The results show that Moringa *oleifera* leaf extract (low dose) and Boo-Sta Plus caused a significant increase ($p < 0.05$) in the RBC, Hb, PCV, whereas a high dose of the leaf extract caused a decrease in the red blood cell indices when compared with the control. The administration of extract caused weight increase at a high dose comparatively to the Boo-Sta Plus and control. It is therefore concluded that ethanol extract of *Moringa oleifera* has positive effects on blood parameters and body weight and could serve as a substitute for Boo-Sta Plus thereby curbing the adverse health implications of artificial boosters.

Keywords: Artificial booster, Boo-Sta Plus, dietary fortification, haematological indices, *Moringa oleifera*.

INTRODUCTION.

The need to provide enough protein for consumption and beef up availability of food as a measure to combat food scarcity is on the increase and this drive has put great pressure on those in animal production. To meet up with the increasing demands, many have resorted to food fortification. Fortification of food with natural products in order to boost nutritional quality or to achieve the therapeutic target of the products has really been on the increase at least for the past 10 years (Marinac *et al.,* 2007). The products used for food fortification are called nutraceuticals, herbals, dietary supplements or other functional foods and all of them basically serve to improve the feeble or minimize the risk of diseases through prevention (Chauhan *et al*., 2013). Most of these Nutraceuticals contain bioactive agents that may be antioxidants (Shahidi, 2012).

Fortification of food is not just trending in the human diet but animal diet. Most poultry companies have employed dietary fortification means to achieve better results in meat quality, protein mass, weight gain and other animal husbandry for availability sake or accessibility and cost reduction. Artificial or intergene food/growth booster has often been the first option on the list against WHO and other health related bodies' recommendation (Anwar *et al*., 2007). Boo-Sta Plus is an example of such artificial boosters that have been utilized for the fortification of animal feed. The composition of Boo-Sta Plus is Lysine, methionine, isoleucine, enzyme, herbs, vitamin B complex, $Feso_4$, $Cuso_4$ and molasses. Growth promoters are mainly used in farm animals with the purpose of promoting growth, improving the distribution of fat and protein, and increasing the feed-to-muscle conversion rate. However, some residues may remain in the tissues of the treated animals several days after their administration at toxicologically unacceptable concentrations. Other promoters like β-agonists affect consumers with certain phenomena like gross tremors of the extremities, tachycardia, nausea, headaches, and dizziness. Focus is now being drawn to herbs that can produce the same effect of enhancing meat quality, protein mass and weight gain without the negative effect of artificial boosters. Most growth promoters are orally active and can be administered either in the feed or in the drinking water, while other active hormones can be administered in the form of small implants into the subcutaneous tissue of the ears. This allows for slow release that may last for several weeks or even months and the ears are discarded at the slaughter. Some fraudulent practices consist of the use of mixtures of several substances like β-agonists and corticosteroids at very low amounts in order to obtain a synergistic effect for a similar growth promotion while reducing the probability of detection by official control laboratories. With the increase in the emergence of antibiotic-resistant pathogens, it became necessary to introduce economically viable alternatives to antibiotics. Such alternatives include probiotics, prebiotics, organic acids and their salts and phytogenic additives (a wide range of plants and spices and their derivatives). During the past fifteen years, phytogenic additives in animal nutrition have attracted attention for their potential role as alternatives to antibiotic growth promoters (Puvača *et al.,* 2013).

Moringa Oleifera leaves are rich in protein; the dried leaves contain about 30% protein with all 9 essential amino acids present in various amounts (Moyo *et al*., 2007). The leaves of *M. oleifera* have also been found to contain appreciable amounts of total phenols, calcium, potassium, magnesium, iron, manganese and copper (Hekmat *et al*., 2015). *M. oleifera* leaves are also good sources of phytonutrients such as carotenoids, tocopherols and ascorbic acid (Saini *et al*., 2016; Saini *et al*., 2014d). The leaves also contain rich amounts of vitamins and minerals (Joshi and Mehta, 2010). Minerals contained in moringa leaves include iron, zinc, calcium, phosphorus etc (Moyo *et al.,* 2007). Moringa leaves are rich sources of β-carotene (pro-vitamin A) and it has been reported that consumption of 8 g of the leaf powder will satisfy nearly all the vitamin A needs for a child between 1-3 years (Fuglie, 2011). Due to this rich content of nutrients and phytochemicals, it is used as an alternative source for nutritional supplements and growth promoters in some countries (Anwar *et al*., 2007). *Moringa oleifera* also plays a major role in the health and treatment of diseases, experiments has shown its great potential in reducing blood sugar level in addition to its anti-inflammatory (Cheenpracha *et al*, 2010; Rakesh and Singh, 2011; Mahajan and Mehta,2010) anticancer and antimicrobial effect (Moon *et al*., 2012; Chattopadhyay *et al*., 2011). Beyond the interesting presence of proteins, lipids, and carbohydrates, *M. Oleifera* seeds contain vitamins A and B1 (Mbah *et al*., 2012). They are also sources of minerals, micronutrients, and bioactive compounds such as flavonoids, saponins, sterols, phytates, and trypsin inhibitors. In the last few years, one of the main concerns of the poultry industry is the ban on antibiotic growth promoters by the European Union. These products are now considered human health risk factors for their possible role in the emergence of microbial resistance (Bharali *et al*., 2003). These interesting nutritional qualities of *Moringa Oleifera* make it a ready alternative for artificial growth booster. Consequently, the aims of this work were to investigate the effect of ethanol leaf extract of *Moringa oleifera* and Boo-Sta Plus on the body weight of Wistar albino rats and to investigate the effect of ethanol leaf extract of *Moringa oleifera* leaf and Boo-Sta Plus on the haematological indices of Wistar albino rats.

MATERIALS AND METHODS

Experimental animal

A total of 20 Wistar albino rats weighing 150 – 170 g were used for the study. All the animals were purchased from the animal house of the University of Calabar Pharmacology Department, Cross River State, Calabar. The body weights of the animals were taken weekly before and during the experiments using Mettler-sensitive balance (number 202845). The approved ethical considerations for using experimental animals as specified by the ethics committee of the Cross River University of Technology were duly followed in the course of this experiment.

Experimental design

A completely randomized design was used to assign the animals (both male and female) into four groups of five (5)

rats per cage (group) as shown below:

Group A (Control): Received normal rat feed + drinking water ad libitum daily for 21 days.
Group B (MLLD): Received same as group 1 plus ethanol extract of *Moringa oleifera* (200 mg/kgbw once daily).
Group C (MLHD): Received same as group 1 plus ethanol extract of *Moringa oleifera* (400 mg/kgbw once daily).
Group D (BP): Received same as group 1 plus 2 ml/kg bw of Boo-Sta Plus once daily)

The administration was done orally using metal oropharyngeal cannula and the experiment lasted for 21 days. The animals were allowed to acclimatize to their environment for one week before the commencement of the extract administration.

Collection and identification of leaf sample

Moringa oleifera leaves were harvested from a farm in Ekpo Abasi, Calabar South Local Government Area of Cross River State to the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, Cross River University of Technology, Calabar, Nigeria, for identification. Leaves of *Moringa oleifera* were first dried in the shade and left in ethanol (70%) for more than two days in the Soxhlet apparatus. Then the 70% ethanol extract was dried in Rotary evaporator apparatus, weighed and dissolved in distilled water to give the final concentration.

Preparation of leaf extract

200 g of fresh *M. oleifera* leaf were properly washed with tap water and air-dried. They were further subjected to oven drying for 14 hours at 40^{o}C and were blended to a fine powder using electric blender. The blended *M. oleifera* leaf was weighed out using an electronic weighing balance and was soaked with ethanol (70%) for 72 hours in the Soxhlet apparatus. The 70% ethanol extract was dried in a Rotary Evaporator apparatus, weighed and dissolved in distilled water to give the final concentration of 200 mg extract/kg and 400 mg extract/kg and were administered orally by Gavage for the different groups for 21 days.

Hematological analysis

All rats were sacrificed after 21 days of treatment with the extract of *M. oliefera* leaf and blood samples were collected via cardiac puncture of the experimental rats in capillary tubes coated with ethylene diamine tetra-acetic acid (EDTA). The tubes were immediately capped, kept at –4°C and were immediately analyzed for blood parameters using an automated coagulating Sysmex apparatus of type 8999. The parameters included: Hemoglobin (Hb), Red Blood Cell Count (RBCs), Packed Cell Volume (PCV), and Red Blood Cells (RBC) were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (2002).

Statistical analysis

The data generated were statistically analyzed using analysis of variance (ANOVA) and results were considered to be significant at a p-value less than 0.05 ($p<0.05$). The results were expressed as mean ± standard errors of the mean (SEM) for all values.

RESULTS

The mean value of the effect of leaf extract of *M. oleifera* in rats and the effect of Boo-Sta Plus was compared with the control. Table 1 shows the effect of the administration of ethanol leaf extract of *M. oleifera* on the body weights of Wister albino rats at the beginning and at the end of the experiment. The study showed that there was a significant difference in body weight of animals fed with *M. oleifera* ethanol leaf extract at a high dose (MLLH) of 400 mg/kgbw when compared to the control. Also, rats treated with Boo-Sta Plus also showed a significant increase even though the increase was more pronounced in the animal that had a high dose of the leaf extract.

Table 2 shows the effect of the administration of ethanol leaf extract of *M. oleifera* on the body weights of Wister albino rats and observes the weekly variation in weight.

The mean value of some red blood cell parameters of rats fed daily with Moringa leaves and Boo-Sta Plus for 21 days was the focus of our study in Table 3.

Table 3 shows the effect of the leaf extract of *M. Oleifera* and Boo-Sta Plus on haemoglobin concentration. Following the administration of leaf extract, It was observed that there was an increase in haemoglobin concentration of experimental animals fed with *Moringa Oleifera* at low dose when compared to control, also, there was an increase in haemoglobin concentration of experimental animals fed with Boo-Sta Plus. Whereas those animals fed with high dose of *M. Oleifera* recorded a decrease in HB when compared to control.

Table 3 shows the effect of *M. oleifera* on packed cell volume. Following the administration of the leaf extract, it was observed that there was an increase in PVC value of experimental animals fed with *M. oleifera* at low dose when compared to the control. However, there was a decrease in PCV of the experimental animals fed with *M. oleifera* at high dose when compared with the control even though not significant. There was an increase in the experimental animals fed with Boo-Sta Plus when compared with the control.

Table 1. Mean body weight (g) of rats provided with ethanol extract of *M. oleifera* leaf and Boo-Sta Plus for 21 days in captivity.

Parameter	Initial body weight	Final Body weight	Differences (g)	Differences (%)
Control (g/bw)	157.000 ± 0.20	163.000 ± 0.60	6.0 ± 0.30	3.8
MLLD (g/bw)	178.750 ± 2.43	181.250 ± 5.66	2.5 ± 3.23	1.4
MLHD (g/bw)	164.000 ± 0.20	178.000 ± 0.60	14.0 ± 0.40	8.5
BP (g/bw)	177.000 ± 51.00	185.500 ± 52.50	10.5 ± 1.50	5.9

MLLD = Moringa leaf low dose; MLHD = Moringa leaf high dose; BP = Boo-Sta Plus. P > 0.05 level of significance. Values are expressed as Mean ± SEM.

Table 2. Mean weekly body weight (g) of rats provided with ethanol extract of *M. oleifera* leaf and Boo-Sta Plus for 21 days in captivity.

Parameter	Initial body weight	Wk 1	Wk 2	Final body weight
Control (g/bw)	157.00 ± 0.20	159.00 ± 0.40	160.00 ± 0.60	163.00 ± 0.40
MLLD (g/bw)	178.75 ± 2.43	179.75 ± 0.75	180.75 ± 3.20	181.25 ± 5.66
MLHD (g/bw)	164.00 ± 0.20	168.00 ± 0.22	170.00 ± 0.30	178.00 ± 0.10
BP (g/bw)	177.00 ± 51.00	179.00 ± 48.00	182.50 ± 54.50	187.50 ± 52.50

Table 3. Mean values of red blood cell parameters of rats provided daily with ethanol extract of *M*. *oleifera* leaf and Boo-Sta Plus for 21 days in captivity (mean ± SD).

Parameter	RBC (10^6/mm^3)	HB (g/dl)	PCV (%)
Control	5.80 ± 0.71	11.55 ± 1.05	31.47 ± 4.25
MLLD	7.37 ± 0.08[a]	14.44 ± 1.63	42.45 ± 2.25 [a]
MLHD	4.24 ± 0.07 [a b]	8.80± 0.17[a b]	26.60± 0.27 [a]
BP	6.28 ± 0.14	13.10 ± 0.20	35.90 ± 0.15
Normal Range	6.9 -11.2	10 -14	30 – 48

MLLD = Moringa leaf low dose; MLHD = Moringa leaf high dose and BP = Boo-Sta Plus; RBC = Red blood cell; HGB = Hemoglobin; PCV = packed cell volume; Values are expressed as Mean ± SEM; a = P < 0.05 vs Control; and b = p < 0.05 vs BP.

DISCUSSION

The present study focused on the comparative effect of ethanol extract of *Moringa oleifera* leaf and Boo-Sta Plus on the body weight and red blood cell indices of wistar albino rats. The result obtained showed that the mean values of red blood cell parameters of wistar rats after administration of the ethanol extract of *M. oleifera* leaf at low dose increased significantly. The Boo-Sta Plus also increased red blood cell indices, packed cell volume, haemoglobin concentration of wistar albino rats even though the increase achieved with the low dose of the ethanol extract of *M. oleifera* leaf was more. The increase observed could be due to the presence of amino acids, vitamins and minerals especially iron present in the leaf extract. The phytochemical constituent of the leaf extract is a well-known hemopoietic factors that have direct effect on the production of red blood cells in the bone marrow. This finding are in agreement with the assertion of Joshi and Mehta (2010), as such with *M. oleifera* leaf extract which is a natural booster, the artificial booster like Boo-Sta Plus can be exempted from animal feeds.

The effect of the extract on the rats that received high dose (400 mg/kgbw) of the extract was more than what was observed in those rats that were administered with Boo-Sta Plus. The administration of *M. oleifera* at high dose and Boo-Sta Plus caused a significant increase ($p<0.05$) in body weight gain. The increase in the body weight of rats might be due to the fact that *M. oleifera* is rich in protein, amino acids, vitamins and minerals particularly iron (Faye, 2011). Also, their antibacterial and antifungal property could help the animal stay healthier. The increase in body weight may also be attributed to captivity, where energy expenditure is minimal (Fadi *et al*., 2010). With a better weight gain with *M. oleifera*, the hazards of artificial boosters on the animal can be totally prevented.

The present study also shows that the ethanol extract of *M. oleifera* caused an increase in body weight of wistar albino rats at a high dose and in the rats that received the

Boo-Sta Plus. There was no appreciable change in the body weight of animals that were administered with 200 mg/kgbw low dose of *M. oleifera* leaf extract.

Summary/Conclusion

1. The *M. oleifera* extract is a good source of body booster.(Anwar *et al.,* 2007). At 200 mg/kgbw low dose, the extract of *M. oleifera* affected the heamatological parameter positively while at higher dosed it causes significant increase in weight better that artificial booster.
2. The *M. oleifera* extract had effects on RBC, PCV and HGB of rats that fed on it. It therefore follows that *M. oleifera* is a highly nutritional plant being ideal in developing countries (Zongo, 2013).
3. *M. oleifera* has a good potential for several properties such as nutritional values, amino acids and flavonols content which can be used in food supplement and cosmetic industry.

Recommendation

1. Using the extract of *M. oleifera* helps in body weight boosting at high doses and could be used as an alternative to artificial boosters and could be a better, cheaper and readily available alternative to artificial boosters.
2. At low levels they have positive effect on red blood parameters and are good blood boosters.
3. The target result is what determines the dose at which it will be administered.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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