

Modification of testosterone levels with *Piptadeniastrum africanum* and *Cordia plathytyrsa* may influence metabolic parameters in Wistar rats

Mary-Ann Angie Mbong^{1,*}, Françoise Raïssa Ntentie², Boris Gabin Kingue Azansta¹, Gislain Paka Djiokeng¹, Henriette Thérèse Dimodi³, Janvier Aimé Fotso Youovop¹, Inelle Makamwe¹, Huiny Miriane Fotso Tiénou¹ and Enyong Julius Oben¹

¹ Department of Biochemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon.

² Department of Earth and Life Sciences, Higher Teachers' Training College, University of Maroua, Maroua, Cameroon.

³ Center for Food and Nutrition Research (CRAN), Institute of Medical Research and Medicinal Plants Study (IMPM), Ministry of Scientific Research and Innovation, Yaounde, Cameroon.

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Abstract

Testosterone supplementation in men regularizes its levels and this is thought to ameliorate body composition and thus metabolic parameters. The aim of this study was to evaluate the effect of methanolic extracts of *Piptadeniastrum africanum* and *Cordia plathytyrsa* on serum testosterone level and some parameters of the metabolic syndrome in adult male rats. Methanolic extracts of leaves of *C. plathytyrsa* and bark of *P. africanum* were prepared and used for evaluating antioxidant potential by measuring the polyphenolic content (Folin - Ciocalteu method), the ferric reducing antioxidant power (FRAP) and the scavenging effects of extracts on 1, 1-Diphenyl-2-Picrilhydrazyl (DPPH) and 2,2'azinobis(3-ethylbenzotiazoline-6-sulfonic acid) diammonium salt (ABTS) free radicals. Both extracts of *C. plathytyrsa* and *P. africanum* (150 and 300 mg/kg.BW) were administered orally to male albino rats of 6 to 7 months old. At the end of an experimentation period that lasted 15 days, they were sacrificed, and their blood collected in tubes with and without anticoagulant for preparation of plasma and serum respectively. The prepared samples were used for the evaluation of their lipid profile (triglyceride and total cholesterol), dihydrotestosterone level, antioxidant status (plasma thiols and FRAP), markers of hepatic toxicity (transaminases activities and serum proteins) and a marker of renal toxicity (creatinine). The results obtained showed that both extracts increased testosterone levels as treated rats were found to have significantly higher testosterone levels (by up to 93%) ($p < 0.05$) compared to control. Both extracts did not induce oxidative stress. They also did not modify total cholesterol, glycemia, serum protein levels and evaluated markers of hepatic toxicity. Contrarily, rats treated with *P. africanum* had higher triglyceride levels ($p < 0.05$) compared to control. Those treated with the higher dose of *C. plathytyrsa* had higher levels of creatinine. From the results obtained it was concluded that methanolic extract of *C. plathytyrsa* enhanced testosterone synthesis but did not influence all metabolic parameters evaluated.

Keywords: Testosterone; Metabolic disorders; Plant extracts; Non-communicable diseases

1. Introduction

In men, bioavailable and free testosterone levels decline with age [1]. This decrease accompanies changes in body composition including; increases in fat mass and decreases in lean body mass, disorders in glucose and lipid metabolism [2]. Metabolic disorders generated as a result of changes in body composition have been found to be related to non-communicable diseases like cardiovascular diseases, type 2 diabetes, and cancers [3, 4].

* Corresponding author: Mary-Ann Angie Mbong
Department of Biochemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon.

High levels of testosterone may affect the pathogenesis of age-related diseases or their components by increasing muscle mass, decreasing abdominal obesity, and improving insulin sensitivity [5]. Testosterone replacement therapy in older men with low serum testosterone levels increases lean body mass and decreases fat mass, total cholesterol, and low-density lipoprotein (LDL) without affecting high-density lipoprotein (HDL), all of which may be associated with a decreased risk of cardiovascular diseases [6,7].

Cordia plathytyrsa is known worldwide for its good quality timber. In African traditional medicine, *Piptadeniastrum africanum* stem bark is used to treat abdominal pain, fever, and cough. Its leaves and fruits are eaten as aphrodisiac and tonic. In Cameroonian traditional medicine, both *P. africanum* and *C. plathytyrsa* are used as sex stimulants and are thought to improve both physical performance and male fertility. They are used as additives in palm wine and sometimes in spiritus like whiskeys and vodkas. Whether these plants affect male physical performance and fertility by increasing testosterone levels and whether they have an effect on metabolic parameters through the modification of body composition is unknown. The objective of this work was thus to determine the effect of methanolic extracts of *P. africanum* and *C. plathytyrsa* on testosterone levels and some biochemical markers in adult male *Wistar* rats.

2. Material and methods

2.1. Identification of plants and preparation of extracts

The plant material used in this study included the bark of *P. africanum* and the leaves of *C. plathytyrsa* which were identified and harvested by botanists of the Department of Plant Biology and Physiology, of the University of Yaounde I. They were dried till obtainment of constant weight, then grinded and stored at room temperature.

Preparation of methanolic extracts of bark of *P. africanum* and leaves of *C. plathytyrsa* took place in the Laboratory II of the Department of organic chemistry of the University of Yaounde I. For every 50 g powdered plant material, 250 ml of 90% methanol was used. Maceration was done for 48 hours after which obtained filtrates were concentrated using a rotor vapor and dried in an oven at 40°C until complete evaporation of the solvent. The extracts were then stored at 4°C for further use.

2.2. Antioxidant activity of extracts

Polyphenolic content of extracts was determined using the Folin-Ciocalteu method [8]. The scavenging effect of extracts on DPPH (1, 1-Diphenyl-2-Picrilhydrazyl) free radical and ABTS (2,2'-azinobis(3-ethylbenzotiazoline-6-sulfonic acid) diammonium salt was determined by the method of Katalinić *et al.* [9] and Re *et al.* [10] respectively. The Ferric Reducing Antioxidant Power (FRAP) was determined by the method of Benzie and Strain [11].

2.3. Animal study

25 adult male albino wistar rats weighing between 250 and 300g, aged between 6 and 7 months reared in the animal house of the Laboratory of Nutrition and Nutritional Biochemistry of the Department of Biochemistry of the University of Yaounde I were used in this study.

The animals had free access to standard commercial diet as well as water and were maintained at room temperature conditions. The animals were divided randomly in five groups of five rats each. They were intubated with corresponding plant extracts and concentrations daily for 15 days but those of the control group were intubated with distilled water. The following groups were constituted for the study.

- Cp 150: Methanolic extracts of *C. plathytyrsa* at a dose of 150 mg/kg body weight.
- Cp 300: Methanolic extracts of *C. plathytyrsa* at a dose of 300 mg/kg body weight.
- Pa 150: Methanolic extracts of *P. africanum* at a dose of 150 mg/kg body weight.
- Pa 300: Methanolic extracts of *P. africanum* at a dose of 300 mg/kg body weight.
- Control: Distilled water (Control Group).

At the end of the 15 days experimentation period, the rats were fasted for 12 hours at the end of which glycemia was determined with capillary blood (collected from the tail) using SD Check Blood Glucose Monitoring system by Standard Diagnostic Inc.

The rats were sacrificed after light anesthesia and blood collected by cardiac puncture into EDTA and dry vacutainer tubes for preparation of their plasma and serum respectively. Collected blood was centrifuged at 3400 rpm for 15

minutes after which the supernatant was transferred into pre-labelled 1.5 ml Eppendorf tubes then stored at -20°C for further studies.

2.4. Biochemical analyses

Two parameters of the lipid profile notably plasma total cholesterol and triglycerides levels were determined by the Enzymatic-Colorimetric Methods respectively [12, 13]. The method of Bassett [14] was used to determine serum Dehydrotestosterone (DHT) level using a DHT ELISA kit manufactured by Alpha Diagnostic Int, San Antonio, USA. Creatinine level was assessed according to the protocol of Bartels *et al.* [15]. Plasmatic transaminases levels were determined with the method of Reitman and Frankel [16] while serum protein levels were assessed using the method of Lowry *et al.* [17]. Plasma Thiol levels using the method of Ellman *et al.* [18] were also determined and the method of Benzie and Strain [11] was used to evaluate ferric reducing antioxidant power of plasma.

2.5. Statistical analysis

Results were analyzed with SPSS version 20.0, SPSS Inc. for Windows and expressed as mean±SD. The least significant difference test associated to a one-way analysis of variance were used to compare means between groups. p value was set at 0.05. Microsoft Excel spreadsheet 2016 was used to plot graphs.

3. Results

3.1. *In vitro* and *in vivo* evaluation of antioxidant potential

Both methanolic extracts of *P. africanum* and *C. plathytyrsa* contained polyphenols and antioxidants (Table 1). Polyphenols were more abundant in *P. africanum* which also exhibited better scavenging activities towards ABTS (186.66 ± 1.08 mgEqvitC/g extract) and DPPH (61.82 ± 1.43 mgEqCat/g extract) free radicals compared to *C. plathytyrsa*. Concerning FRAP there was no significant difference between both extracts even if activity was higher with *P. africanum*.

Table 1 Polyphenolic content and antioxidant potential of plant extracts

| | Polyphenol content (mgEqCat/g extract) | FRAP (mgEqCat/g extract) | DPPH (mgEqCat/g extract) | ABTS (mgEqvitC/g extract) |
|--------------------------------------|---|-----------------------------|-----------------------------|---------------------------------|
| <i>Piptadeniastrum africanum</i> | 402.34 ± 6.03 ^a | 36.59 ± 0.12 ^a | 61.82 ± 1.43 ^a | 186.66 ± 1.08 ^a |
| <i>Cordia plathytyrsa</i> | 54.90 ± 6.78 ^b | 28.28 ± 0.73 ^a | 15.71 ± 0.53 ^b | 70.82 ± 1.61 ^b |

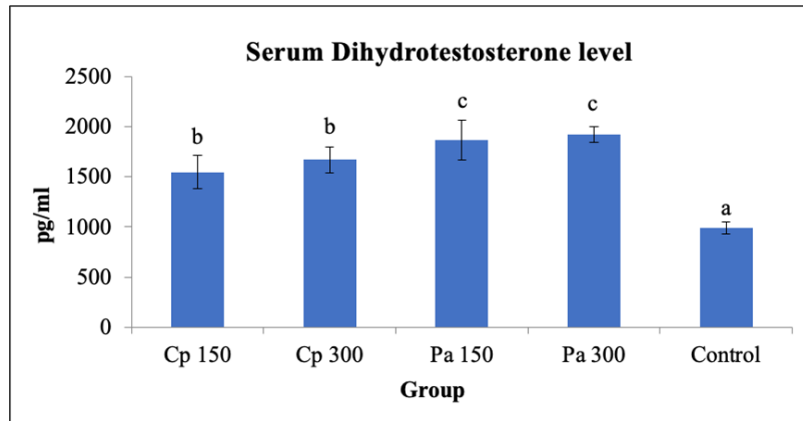
In the same column, values with the different letters were significantly different at p <0.05

3.2. Effect of treatment on testosterone levels

At the end of the experimentation period there were significantly higher DHT levels in all groups of rats when compared to the control group (p≤0.0001) (Figure 1). The highest levels were observed in groups treated with extract of *P. africanum*. This implied that methanolic extracts of *P. africanum* and *C. plathytyrsa* increased testosterone levels in *Wistar* rats at the end of an experimentation period of 15 days.

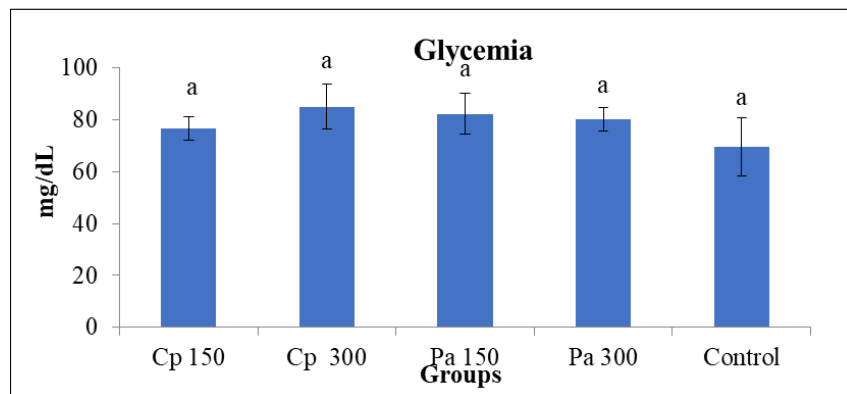
Figure 2 shows blood glucose levels in rats at the end of the fifteen days experimental period. Blood glucose was found to be similar in all groups (p>0.05).

As can be observed in Figure 3, total cholesterol levels were found to be similar in all treated groups (p>0.05) when compared to the control group meanwhile serum triglyceride levels were higher in rats treated with extracts of *P. africanum* (p≤0.01). This was not the case with rats treated with extracts of *C. plathytyrsa* in which triglyceride levels were found to be similar (p>0.05) to that of the control group.



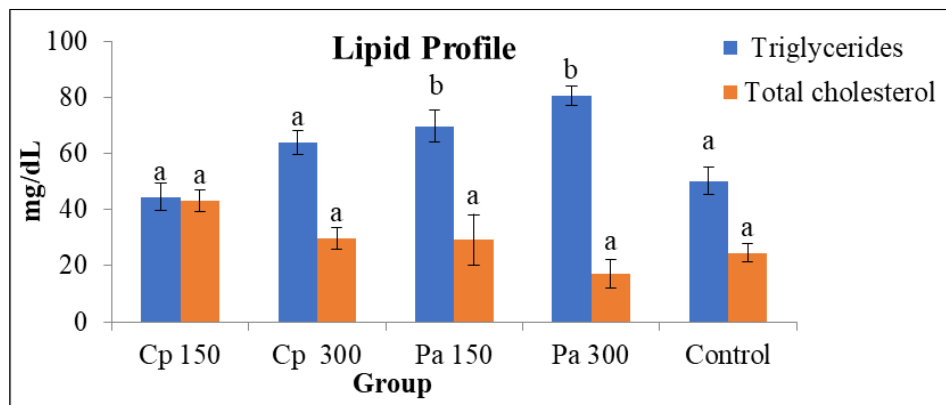
Data were expressed as Mean \pm SEM; Cp 150= group of rats that received 150mg/kg.bw of *C. plathytyrsa* daily; Cp 300= group of rats that received 300mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150mg/kg.bw of *P. africanum* daily; Pa 300= group of rats that received 300mg/kg.bw of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$)

Figure 1 Serum DHT levels of rats after experimentation



Data is expressed as Mean \pm SEM. Cp 150= group of rats that received 150mg/kg.bw of *C. plathytyrsa* daily; Cp 300= group of rats that received 300mg/kg.BW of *C. plathytyrsa* daily; Pa 150= group of rats that received 150mg/kg.BW of *P. africanum* daily; Pa 300= group of rats that received 300mg/kg.BW of *P. africanum* daily; Control= group of rats that received distilled water daily

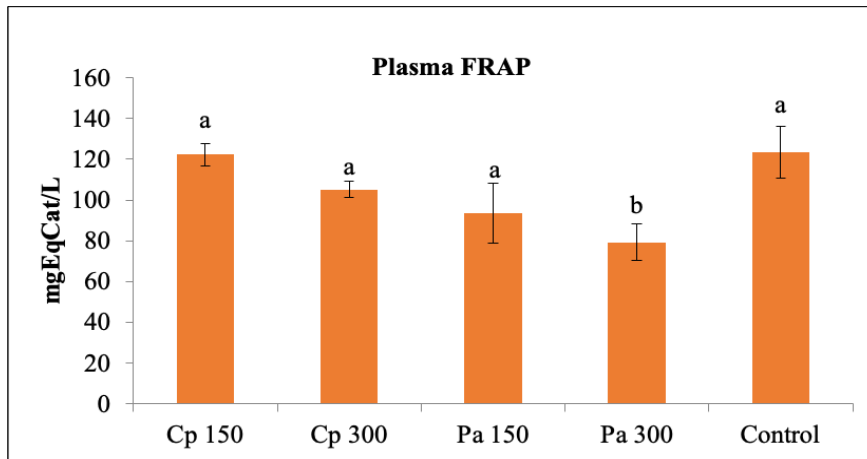
Figure 2 Blood glucose level of rats at the end of the experimental period



Data are expressed as Mean \pm SEM. Cp 150= group of rats that received 150 mg/kg.bw of *C. plathytyrsa* daily; Cp 300= group of rats that received 300mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150 mg/kg.bw of *P. africanum* daily; Pa 300= group of rats that received 300 mg/kg.bw of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$)

Figure 3 Triglyceride and total cholesterol levels after experimentation

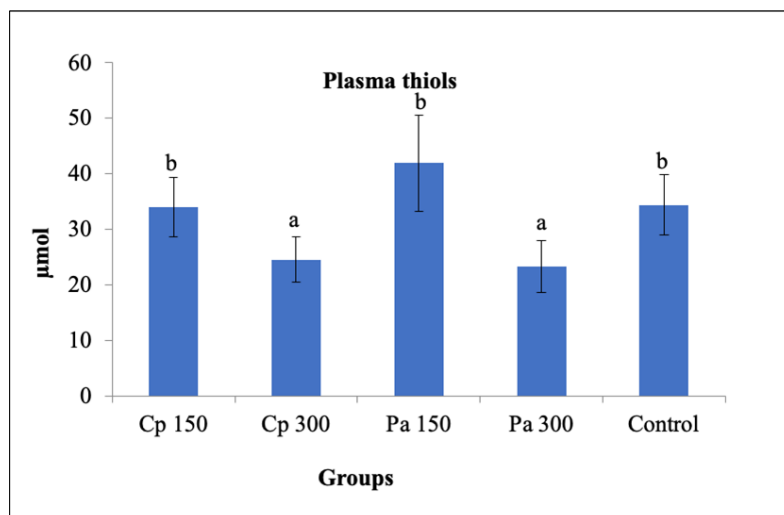
Figure 4 represents plasmatic antioxidant potential of rats after treatment with extracts. No significant difference was found between control group and extract-treated groups except for the group of rats treated with 300mg/kg.bw of *P. africanum* where FRAP level was significantly lower.



Data are expressed as Mean \pm SEM. Cp 150= group of rats that received 150 mg/kg.bw of *C. plathytyrsa* daily; Cp 300= group of rats that received 300 mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150mg/kg.bw of *P.africanum* daily; Pa 300= group of rats that received 300mg/kg.BW of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$)

Figure 4 Ferric reducing antioxidant power of plasma at the end of the experimentation

The plasmatic thiol levels of rats were found to vary with dose for both extracts as compared to the control group. As can be noticed in Figure 5, the lower dose (150 mg/kg.bw) of both extracts portrayed levels that were similar to the control group ($p > 0.05$).

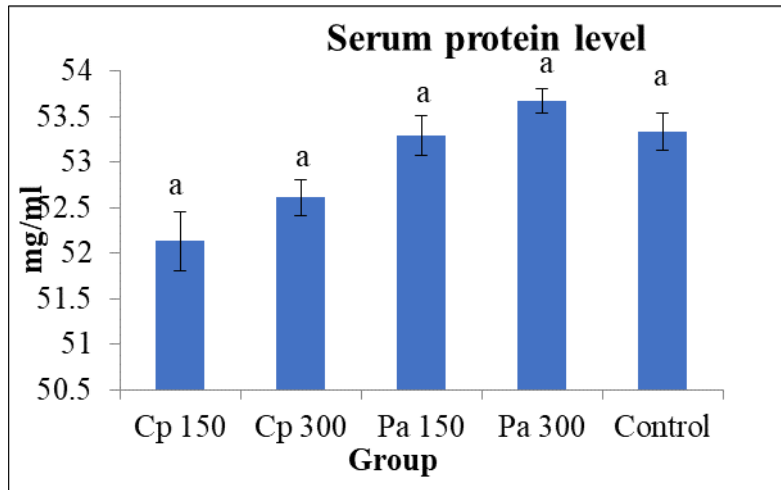


Data are expressed as Mean \pm SEM. Cp 150= group of rats that received 150 mg/kg.BW of *C. plathytyrsa* daily; Cp 300= group of rats that received 300 mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150 mg/kg.BW of *P. africanum* daily; Pa 300= group of rats that received 300 mg/kg.bw of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$)

Figure 5 Plasma thiols levels in experimental rats

3.3. Effect of treatment on toxicity markers

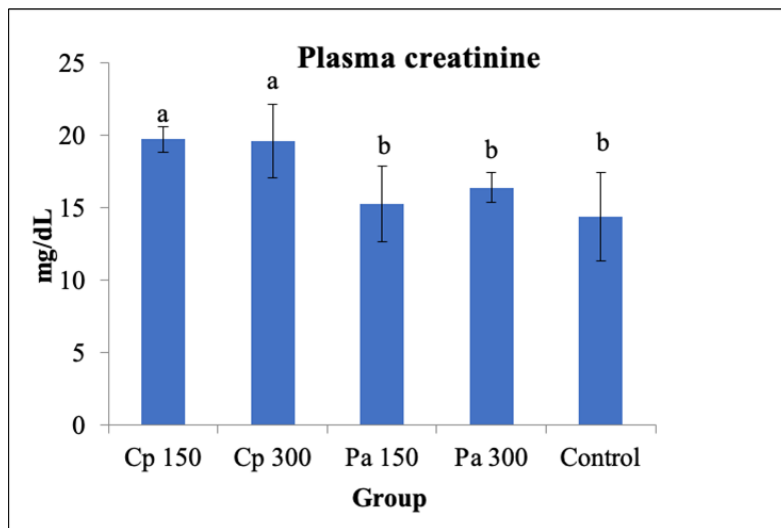
Serum protein levels are reported in Figure 6. Treatment of rats with *P. africanum* and *C. plathytyrsa* did not lead to any significant modification of blood protein level ($p > 0.05$).



Data are expressed as Mean \pm SEM. Cp 150= group of rats that received 150 mg/kg.BW of *C. plathytyrsa* daily; Cp 300= group of rats that received 300 mg/kg.BW of *C. plathytyrsa* daily; Pa 150= group of rats that received 150 mg/kg.BW of *P. africanum* daily; Pa 300= group of rats that received 300 mg/kg.BW of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$).

Figure 6 Serum Protein level of experimental rats

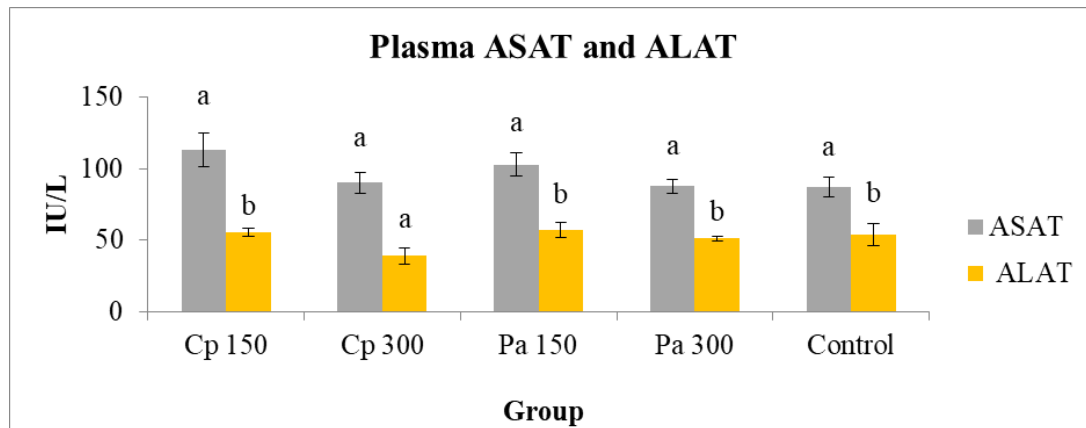
The Creatinine plasmatic levels were found to be similar between rats treated with *P. africanum* and control group while those treated with *C. plathythyrsa* showed higher levels at both doses compared to the control group.



Data are expressed as Mean \pm SEM; Cp 150= group of rats that received 150 mg/kg.bw of *C. plathytyrsa* daily; Cp 300= group of rats that received 300 mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150 mg/kg.BW of *P. africanum* daily; Pa 300= group of rats that received 300 mg/kg.bw of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letters show significant difference between groups ($p < 0.05$).

Figure 7 Creatinine levels in experimental rats

The level of transaminases measured in plasma was similar among groups receiving *P. africanum* at both doses and *C. plathytyrsa* at the 150 mg/kg.bw dose compared to the control group. Concerning rats treated with *C. plathytyrsa* at 300 mg/kg.bw lower alanine amino transferase (ALT) levels were obtained ($p < 0.05$) (Figure 8).



Data are expressed as Mean \pm SEM. Cp 150= group of rats that received 150 mg/kg.bw of *C.plathytyrsa* daily; Cp 300= group of rats that received 300 mg/kg.bw of *C. plathytyrsa* daily; Pa 150= group of rats that received 150 mg/kg.bw of *P. africanum* daily; Pa 300= group of rats that received 300 mg/kg.bw of *P. africanum* daily; Control= group of rats that received distilled water daily. Different letter shows significant difference with other treated groups ($p < 0.05$)

Figure 8 Transaminase levels in the plasma of experimental rats

4. Discussion

Testosterone is the primary male sex hormone that is vital for sustaining proper erectile function and libido. It is also critically involved in muscle building, burning fat, and supporting endothelial function, energy level, mood, immune function, and bone density. Research suggests that low testosterone levels may be intimately linked to non-transmissible diseases [19, 20]. Research suggests that 20-64% of men with diabetes have low testosterone levels; older men appear to be particularly more susceptible [21]. Likewise, low serum testosterone is common amongst men with obesity related diseases and some scientists have proposed that low testosterone might serve as a prognostic tool for early detection of obesity related metabolic conditions [19]. Restoring testosterone to youthful ranges in older men may confer a wide array of benefits to men affected by abdominal obesity and type II diabetes. The reported benefits of testosterone administration in these men include improved glucose homeostasis, reduction of abdominal fat and improved erectile function [5]. Likewise, scientists believe that testosterone replenishment may help reverse some key biochemical abnormalities that underlie the metabolic syndrome, such as insulin resistance and central obesity [22]. The management of low testosterone levels makes mention of a multi-faced approach that includes proper lifestyle, nutrition, dietary modifications, exercise and nutritional supplements [23]. Research on herbal supplements has uncovered numerous examples of plant extracts that overcome testosterone deficiency by inhibiting aromatization and or increasing production naturally. In Cameroonian traditional medicine, *P. africanum* and *C. plathytyrsa* are used as sex stimulants. This study which was aimed at investigating the effects of these plants on serum testosterone levels and some metabolic markers showed that extracts of *P. africanum* and *C. plathytyrsa* had androgenic activity as they increased serum testosterone levels. Other herbal extracts such as those from *T. foenum-graecum* and *W. somnifera* have been shown to increase blood testosterone levels [24, 25]. The presence of certain bio-active molecules in extracts could be responsible for this enhancement. Saponins, sterols and triterpenes are some groups of bio-active molecules that are thought to have the ability of boosting testosterone synthesis [26, 27]. Higher testosterone levels were obtained with *P. africanum* compared to *C. plathytyrsa*. The difference could be due to the presence of more testosterone boosting molecules in *P. africanum* or simply more active molecules. In this study, the extracts were not analyzed for molecules like saponins and triterpenes that are known to be good testosterone enhancers [27]. These molecules are thought to act by ameliorating the activity of enzymes of the testosterone production pathway [28] or by increasing the concentration of the luteinizing hormone (LH) [29].

The multiple risk factor syndrome know as metabolic syndrome is a growing medical problem in developed as well as developing countries [30]. Elevated levels of serum triglycerides were found in rats treated with both doses of *P. africanum* compared to non treated rats while those treated with *C. plathytyrsa* showed no difference. This could be due to the fact that *P. africanum* increased testosterone levels better than *C. plathytyrsa*. Studies have shown that high testosterone levels induce the liberation of free fatty acids in adipose tissue which are substrates of triglyceride synthesis in the liver [31]. On the other hand, glycemia and total cholesterol level were not modified by both extracts, this makes them good candidates for testosterone enhancement.

Since testosterone increases metabolic rate [32] that is known to favor the elevation of oxidative stress, a plant extract that can enhance testosterone synthesis with an additional antioxidant activity could be of great interest. Antioxidant analyses of plant extracts using four different methods (Folin- Ciocalteu, FRAP, ABTS and DPPH methods) showed the presence of important amounts of antioxidants in both extracts. This goes in line with many plant studies that have indicated the presence of antioxidants in plant extracts, like the analyses of 14 herbs and spices that were carried out by Agbor [33]. The evaluation of plasma thiols showed that the lower doses of both extracts maintained these levels to normal. Also total FRAP measured in the plasma showed that except for the higher dose of *P. africanum*, all other treatments maintained antioxidant potential of plasma to normal since their values were comparable to those of the control group. *In vitro* antioxidant evaluation revealed that *P. africanum* had more potential than *C. plathytyrsa* but *in vivo*, *C. plathytyrsa* could be considered as a better antioxidant at least for the methods exploited. It is well known that many parameters affect antioxidant potential.

The increased activities of transaminases ALT and AST, may be an indicator of liver damage [34]. Transaminase activities were not increased by treatment as compared to the control. They were even found to be lower in the group treated with the 300 mg/kg.BW dose of *C. plathytyrsa*. Similar results were obtained with the evaluation of serum proteins levels; they were not lower in treated rats. It has been shown that low levels of serum proteins are early symptoms for the liver toxic injury [35]. For the tests carried out, both extracts, administered at both doses for the experimental period did not cause deleterious effects on the liver. An increase in serum creatinine is associated to renal dysfunction [36]. Serum creatinine in treated rats was seen to be higher in rats treated with *C. plathytyrsa* while levels in *P. africanum* treated rats were comparable to control group levels.

5. Conclusion

Fifteen days treatment of rats with *C. plathytyrsa* and *P. africanum* enhanced testosterone production and did not expose rats to oxidative stress. However, their effect on metabolic parameters were not quite perceptible and obviously more experiments need to be done to investigate this.

Acknowledgments

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Author's contribution

MAM-A and OJE designed the study, NFR and ABK analyzed the data, NFR, MAM-A, and YJA wrote the manuscript, DPG, DHT, MI and FTHM carried-out the experimentations and biochemical analyses.

Disclosure of conflict of interest

The authors in this paper have no of conflict of interest.

Statement of ethical approval

The experimental protocol and the maintenance of the laboratory animals were carried out in accordance with the standard ethical guidelines for the use and care of laboratory animals as described by Joint Institutional Review Board for Animal and Human Bioethics, CRFD-SVSE of the University of Yaounde I.

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