Scientific Research Publishing

Open Journal of Applied Sciences, 2022, 12, 1284-1295
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Elucidation of the Probable Ovarian-Dependent Mechanism of the Estrogenic Effects of *Buccholzia coriacea* and Progesterone Effects of *Cogniauxia pololeana* in the Rat

Bonaventure Max Lazare Peneme, Arnaud Wilfrid Etou Ossibi, Hermann Akassa, Radard Ondélé, Ange Antoine Abena

Laboratory of Biochemistry and Pharmacology, Faculty of health Sciences, Marien Ngouabi University, Brazzaville, Republic of the Congo
Email: etouarnaud@yahoo.fr

How to cite this paper: Peneme, B.M.L., Ossibi, A.W.E., Akassa, H., Ondélé, R. and Abena, A.A. (2022) Elucidation of the Probable Ovarian-Dependent Mechanism of the Estrogenic Effects of *Buccholzia coriacea* and Progesterone Effects of *Cogniauxia pololeana* in the Rat. *Open Journal of Applied Sciences*, **12**, 1284-1295.
https://doi.org/10.4236/ojapps.2022.127088

Received: June 21, 2022
Accepted: July 25, 2022
Published: July 28, 2022

Abstract

The present study was carried out with the objective of evaluating, in castrated rats, the utero trophic, hormonal and biochemical activities of aqueous extracts of *Buchholzia coriacea* (BC) and *Cogniauxia podolaena* (CP) leaves. Each extract administered at the dose of 600 mg/Kg in castrated rats did not cause a significant change in the fresh weight/dry weight ratio of the uterus compared to castrated rats given distilled water. However, those receiving 17-β-estradiol as a reference product showed a significant ($p < 0.5$) increase in this ratio. These results indicate the absence of uterotrophic effects of both extracts in the ovariectomized rat compared with the effects of 17-β estradiol. In addition, the extracts did not cause significant changes in estrogen or progesterone levels in treated rats, as observed with 17-β-estradiol. In addition, the determination of protein and total cholesterol in the uterus of castrated rats treated with each extract did not show significant variation from controls. At the time, castrated rats treated with 17-β-estradiol showed a significant increase ($p < 0.5$) in uterine protein level and a significant decrease ($p < 0.5$) in total cholesterol level. Only the blood protein level was significantly increased in the castrated rats that received the extracts. These results suggest that the respective estrogenic and progesterone effects of the extracts of the two plants may be ovarian-dependent, these plants would not contain phytohormones.

Keywords

Castrated Rat, *Cogniauxia podolaena*, *Buchholzia coriacea*, Ovary-Dependent

1. Introduction

In Congo Brazzaville, traditional medicine uses plants to cure different kinds of diseases and prevent pregnancy; without however having scientific data on their pharmacological efficacy and mechanism of action. According to [1], two plants in the traditional Congolese pharmacopoeia, namely *Buchholzia coriacea* Engl (Capparidaceae) and *Cogniauxia podolaena* Baill (Cucurbitaceae) are frequently used by the population for their curative virtues.

In addition, the literature reports that the decoction *of Cogniauxia podolaena* leaves is used in Brazzaville to treat infertility [2]. Also [3] [4] reported that the plant drug from the leaves of *Cogniauxia podolaena* is forbidden to pregnant women because of its abortifacient power. As for *Buchholzia coriacea*, it is reportedly used in traditional Nigerian medicine for the treatment of amenorrhoea [5].

Pharmacological studies carried out on the aqueous extracts of *Cogniauxia podolaena* have highlighted their anti-diabetic [6], anti-plasmodial and cytotoxic [7], and analgesic [8] activities. *Buchholzia coriacea* extracts have also been studied in the laboratory for anti-inflammatory, analgesic and antipyretic [9], antibacterial and antifungal [10], hypoglycaemic and antioxidant activities [11].

According to [12] [13] *Buchholzia coriacea* and *Cogniauxia podolaena* block the sexual cycle of the rat at the estrus and di-estrus stages respectively, thus demonstrating their oestrogenic and progesterone effects in mammals. It is known that sex hormones are essentially produced by the ovaries; administration of these hormones in the absence of the ovaries makes it possible to correct certain ovarian deficiencies in castrated animals. It is in this context that the present study was conducted, which consists of evaluating the effects of aqueous extracts of the leaves of *Buchholzia coriacea* and *Cogniauxia podolaena* in castrated rats in order to elucidate the probable ovary-dependent mechanism of action of these two plants.

2. Material and Methods

2.1. Plant Material

Leaves of *Buchholzia coriacea* Engl. (Caparridaceae) and *Cogniauxia podolaena* Baill (Cucurbitaceae) were collected in Brazzaville in 2017, and authenticated with the herbarium n˚ 2456 of 17 February 1968 collected by Bouquet; with the herbarium n˚ 548 of 4 August 1963 deposited by Neré. After harvesting, these leaves were cleaned and then dried for three (3) weeks at the Laboratory of Biochemistry and Pharmacology of the Faculty of Health Sciences in Brazzaville at an ambient temperature of 25˚C to 27˚C.

2.2. Animals Used

Virgin wistar rats aged 18 to 22 weeks with an average weight of 100 g were used to evaluate the uterotrophic, hormonal and biochemical activities of the aqueous extracts of the leaves of *B. coriacea* Engl. or *C. podolaena*. These animals came

from the animal house of the Faculty of Science and Technology of the University Marien Ngouabi (Brazzaville-Congo). They were subjected to standard conditions of average temperature varying between 27˚C - 29˚C, photoperiod cycle of 12 hours of light/darkness. They received standard feed and tap water ad libitum.

2.3. Preparation of Plant Extracts

The aqueous extracts used in this study were prepared by decoction, a method of preparation used by most traditional healers: 100 g of pulverised dry leaves of *B. coriacea* or *C. podolaena* were boiled for 15 min in 1000 ml of distilled water. After filtration, the solution obtained was evaporated at 70˚C, using a Heidolph type heater for 48 h; the concentrate obtained was collected in powder form; and the yield was determined. Dissolving 1 g of the concentrated powder in 10 ml of distilled water gave a 100 mg/ml concentrated solution, which was used as an aqueous extract for administration to the rats.

2.4. Evaluation of Uterotrophic Activity of Aqueous Extracts of *B. coriacea* Engl. and *C. podolaena* Baill. Leaves in Castrated Rats

Uterotrophic activities were performed according to the method described by [14] [15] [16] [17] [18].

2.4.1. Ovariectomy in the Rat and Constitution of Batches for Testing

Bilateral ovariectomy was performed on each rat to assess the effect of each plant extract on the uterus and sex hormones in the absence of the ovaries.

Thus, five (5) uncastrated virgin rats and twenty (20) castrated virgin rats divided into five (5) batches of five (5) animals, received each morning during one week; respectively;

By mouth:

- Distilled water (0.5 ml/100g), control for batch 1 (uncastrated rats),
- Distilled water (0.5 ml/100g), control for batch 2 (castrated rats),
- *Cogniauxia podolaena* extract (600 mg/kg), for batch 4 (castrated rats),
- *Buchholzia coriacea* extract (600 mg/kg), for batch 5 (castrated rats).

By subcutaneous route:

- 17-β-estradiol, (100 µg/kg), reference product for batch 3 (castrated rats).

2.4.2. Evaluation of the Live Weight of Rats Treated with Each Extract

The weight of ovariectomized and whole (cycled) rats treated with each extract was recorded daily using a precision balance and compared to control rats.

2.4.3. Assessment of Uterine Weight of Rats Treated with Each Extract

The ratio of fresh to dry uterine weights of the rats treated with each extract was determined to assess the activity of each extract on uterine weight.

Thus, (24 h) after the last administration of the products, the animals were sacrificed by cervical dislocation and the uterus carefully removed. The uterus

was separated from the fatty adhesions and weighed using a Highland Adam precision balance with a capacity of 120 g and a sensitivity of 0.001 g to obtain the fresh weight of the uterus.

The uterus was then placed in an oven for 24 hours at 100˚C and reweighed to obtain the dry weight of the organ. The fresh and dry weights obtained were used to determine:

- The change in the ratio of fresh to dry weight of the uterus in each batch;
- The variation in fresh weight of the uterus between the different batches;
- The variation of the dry weight of the uterus between the different batches.

2.4.4. Evaluation of the Chemical Composition of the Treated Rat Uterus

The 10% uterine homogenates were prepared by grinding the dry uterus in a Potter with 0.9% NaCl solution. The crushed solution was centrifuged at 4000 rpm for 60 min. The supernatant obtained after centrifugation was stored in the freezer at −4˚C; in 1.5 ml Eppendoff tubes for the determination of total cholesterol and protein in the uterus.

2.5. Evaluation of Sex Hormone Levels and Biochemical Parameters of Castrated Rats Treated with *B. coriacea* Engl. and *C. podolaena* Baill. Extracts

Hormonal and biochemical assays were carried out according to the methods described by [15] [19] [20]. Blood samples were taken at each stage of the sexual cycle; in the control and treated rats with each extract in order to compare the variation in sex hormone levels (oestrogen, progesterone) and biochemical parameters (proteins, total cholesterol) with respect to the controls.

Blood from each rat (castrated or not) was gently collected from the ophthalmic vein using Vitrex heparin tubes. After centrifugation of the blood at 4000 rpm for 30 min, the collected plasma was stored in a freezer at −4˚C in 1 ml Eppendoff tubes for the determination of hormones and biochemical parameters.

Vaginal smears using the Haris Shorr technique, described by [20], were only performed in cycled rats as ovariectomized rats have a blocked sexual cycle. Vaginal smears, blood sampling and administration of extracts were performed according to the scheme shown in **Figure 1** below.

2.5.1. Techniques for the Determination of Oestradiol and Progesterone

The assays for oestradiol and progesterone were performed using the Cypress Diagnostics method. The Cypress Diagnostics Estradiol (or Progesterone) ELISA

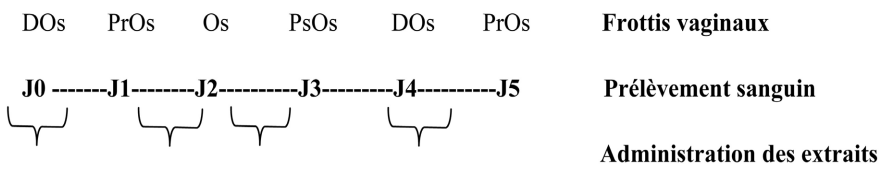

Figure 1. Diagram of vaginal smears, administration of extracts and blood sampling. DOs: di-œstrus; PrOs: pro-œstrus; Os: œstrus; PsOs: post-œstrus.

is an enzyme immunoassay for the quantitative determination of estradiol (or progesterone) in serum or plasma.

2.5.2. Blood and Uterine Cholesterol and Protein Assays

1) Serum and uterine total cholesterol assay

This assay is based on an enzymatic method described by Allain *et al.* (1974) [21] by Bayala (2005) [15]. A drop of the sample (blood or homogenate) is placed on the plate and then spread evenly through the spreading layer into the underlying layers. Read the absorbance of the sample and standard against the reagent blank at 546 nm within 60 min of the end of the incubation.

2) Determination of total serum and uterine proteins

Protein determination was performed according to the method of Gornall, described by [22]. In a basic medium, sodium and potassium tartrate form a soluble complex with cupric ions. The addition of a protein displaces the copper complexed with the tartrate to form a violet-coloured copper-protein complex which shows an absorption maximum at 540 nm. The concentration of the protein to be assayed is then deduced.

3. Results

3.1. Uterotrophic Activity of *B. coriacea* (BC) Engl. and *C. podolaena* (CP) Baill

3.1.1. On the Live Weight of the Rats

Figure 2 shows the weight evolution of the rats during the trial in the 5 batches: (RnC + ED, RC + ED, RC + E2, RC + BC and RC + CP). It shows that the castrated control rats showed a greater weight change compared to the non-castrated control rats ($p < 0.05$). The administration of aqueous extracts of *B. coriacea* and *C. podolaena* at 600 mg/kg to castrated rats did not result in any difference in

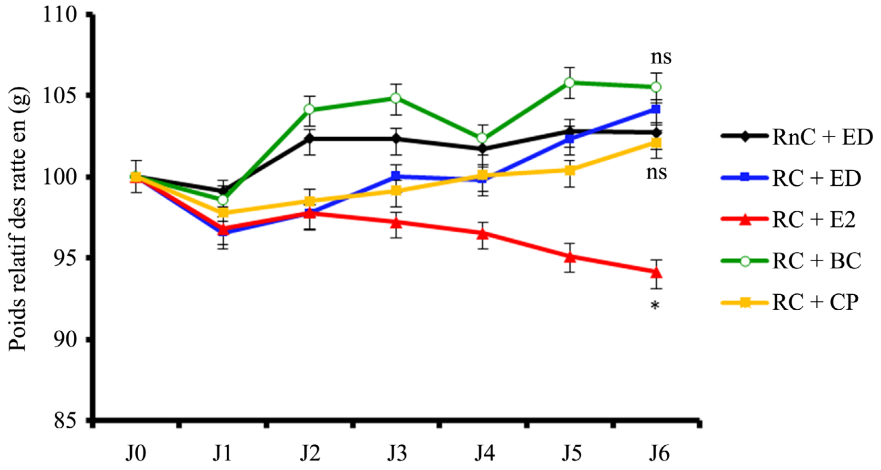

Figure 2. Evolution of live weight of castrated rats treated with *Buchholzia coriacea* and *Cogniauxia podolaena* extract at 600 mg/kg (n = 4). RnC + ED: Uncastrated rats + distilled water; RC + ED: Castrated rats + distilled water; RC + BC: Castrated rats + *Buchholzia coriacea*; RC + CP: Castrated rats + *Cogniauxia podolaena*; RC + E2: Castrated rats + 17β-estradiol; *: Significant difference; ns: Not significant difference.

weight development compared to control castrated rats ($p > 0.05$). In contrast, 17-β-estradiol administered at 100 µg/kg resulted in a significant decrease in the live weight of castrated rats compared to control castrated rats and those treated with each extract ($p < 0.05$).

3.1.2. On Fresh and Dry Weight of the Uterus

Figure 3 and Figure 4 show a fresh and dried uterus respectively. These figures show the significant decrease in size and weight when a uterus is changed from fresh to dry.

Table 1 shows the average fresh uterus weight, the average dry uterus weight and the ratio of fresh to dry uterus weight in each of the 5 batches. It shows that ovariectomy of the rats causes a significant decrease in the ratio of fresh to dry weight of the uterus. Administration of aqueous extract of BC or CP at 600 mg/kg in castrated rats does not cause a change in this ratio. However, administration of 17β-estradiol at 100 µg/kg significantly increased the ratio in castrated rats.

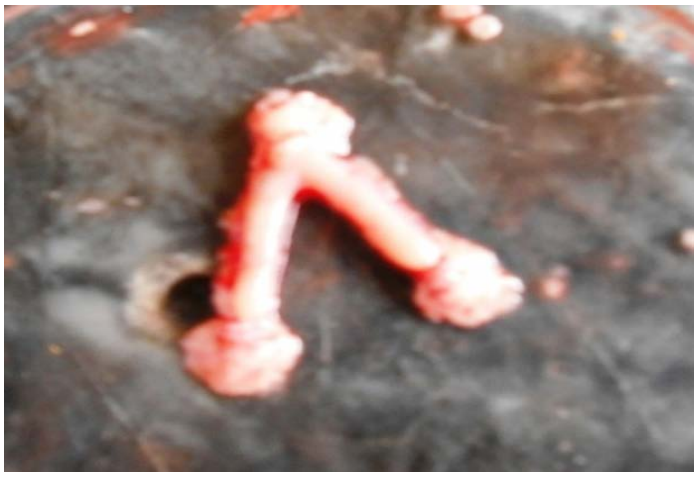

Figure 3. Photograph of a fresh uterus of a castrated rat.

Figure 4. Photograph of a dry uterus of a castrated rat.

Table 1. Fresh weight to dry weight ratio of rat uteri under the effect of aqueous extracts of *Buchholzia coriacea* and *Cogniauxia podolaena* (600 mg/kg).

Traitement	Poids frais utérus (g)	Poids sec utérus (g)	P. frais/P. sec
Lot 1: RnC + ED	0.264 ± 0.08	0.04 ± 0.018	6.6
Lot 2: RC + ED	**0.252 ± 0.051°**	**0.077 ± 0.007ª**	**3.3**
Lot 3: RC + E2	**0.475 ± 0.071***	**0.069 ± 0.005***	**6.9**
Lot 4: RC + BC	0.261 ± 0.006^{ns}	0.074 ± 0.004^{ns}	3.5
Lot5: RC + CP	0.226 ± 0.087^{ns}	0.074 ± 0.024^{ns}	2.9

Values are means ± MSE with n = 4. o: non-significant difference compared to uncastrated rats of the control lot; a: significant difference compared to uncastrated rats of the control lot; *: ($p < 0.05$), significant difference compared to castrated rats of the control lot; ns: non-significant difference compared to castrated rats of the control lot; E2: estradiol; ED: distilled water; BC: *Buchholzia coriacea*; CP: *Cogniauxia podolaena*; RnC: uncastrated rats; RC: castrated rats; P. fresh/dry: fresh and dry weight.

3.2. Uterine Levels of Total Cholesterol and Protein

The uterine concentrations of total cholesterol and protein of the rats after 6 days of treatment and subsequent sacrifice of the animals are presented in Table 2. It indicates that after ovariectomy, uterine protein levels decrease and cholesterol levels increase compared to uncastrated control rats ($p < 0.05$). Administration of aqueous extract of BC or CP at 600 mg/kg, does not cause significant changes in the level of the two biochemical parameters in the uterus of castrated rats. However, 17β-estradiol promotes protein increase and cholesterol decreased in the uterus of castrated rats.

3.3. Sex Hormone Levels and Biochemical Parameters of Castrated RATS Treated with *B. coriacea* Engl. and *C. podolaena* Baill

The blood concentrations of estradiol, progesterone, cholesterol and proteins of the rats according to the phases of the sexual cycle during the test are presented in Table 3.

3.3.1. Estradiol and Progesterone Levels

Table 3 shows that in uncastrated control rats, the mean concentration of estradiol and progesterone in the blood varies with the estrous stage: it is low in di-estrus (10.91 ± 3.20 pg/l and 3.08 ± 0.65 ng/l), increases in pro-estrus (141.60 ± 15.50 pg/l and 4.04 ± 0.21 ng/l), and peaks in the estrus stage (608.60 ± 209 pg/l and 30.50 ± 4.12), before decreasing in post-estrus (103.60 ± 12.10 pg/l and 4.51 ± 0.22 ng/l).

After ovariectomy, the peak levels of estradiol and progesterone decreased significantly in the blood of control castrated rats. Administration of aqueous extract of BC or CP at 600 mg/kg in castrated rats does not result in changes in estradiol or progesterone levels compared to control castrated rats ($p > 0.05$).

Table 2. Protein and total cholesterol levels in the uterus of castrated rats treated with *Buchholzia coriacea* and *Cogniauxia podolaena* extract at 600 mg/kg.

Traitements	Protéines (g/l)	Cholestérol total (mg/dl)
RnC + ED	125.11 ± 3.2	18.53 ± 1.31
RC + ED	**94.12 ± 5.11^{a}**	**25.28 ± 2.22^{a}**
RC + E2	**106.7 ± 3.88***	**21.61 ± 1.18***
RC + BC	92.98 ± 1.39^{ns}	25.15 ± 5.74^{ns}
RC + CP	94.33 ± 3.15^{ns}	24.99 ± 1.91^{ns}

Values are means ± MSE with n = 4. o: non-significant difference compared to uncastrated rats of the control lot; a: significant difference compared to uncastrated rats of the control lot *: $p < 0.05$), significant difference compared to castrated rats of the control lot; ns: non-significant difference compared to castrated rats of the control lot E2: estradiol; ED: distilled water; BC: *Buchholzia coriacea*; CP: *Cogniauxia podolaena*; RC: castrated rats. RnC: non-castrated rats. In bold significant values.

Table 3. Blood levels of estradiol, progesterone, cholesterol and protein in castrated and non-castrated rats treated with *Buchholzia coriacea* and *Cogniauxia podolaena* extracts at 600 mg/kg (n = 5).

Traitements Paramètres		Stades du cycle sexuel			
		Di-œstrus	Pro-œstrus	Œstrus	Post-œstrus
RnC + ED	E2 (pg/ml)	40.91 ± 03.2	141.5 ± 15.5	**608.6 ± 209^{2}**	103.6 ± 12.5
	P4 (ng/ml)	03.08 ± 0.65	04.04 ± 0.21	**30.52 ± 04.1^{2}**	04.51 ± 0.22
	Prot (g/l)	58.48 ± 10.2	71.36 ± 06.4	**143.4 ± 05.4^{1}**	69.12 ± 09.1
	Cho (mg/dl)	73.28 ± 15.2	75.65 ± 12.4	82.63 ± 29.0^{0}	75.33 ± 21.3
RC + ED	E2 (pg/l)	**28.57 ± 12.1^{a}**	**31.36 ± 13.2^{a}**	**50.36 ± 21.2^{b}**	**30.55 ± 18.2^{b}**
	P4 (ng/l)	03.41 ± 12.7^{o}	03.66 ± 02.1^{o}	**05.33 ± 03.1^{a}**	06.71 ± 02.1^{o}
	Prot (g/l)	52.18 ± 10.3^{o}	61.51 ± 05.1^{o}	67.53 ± 99.2^{o}	65.57 ± 03.1^{o}
	Cho (mg/dl)	55.73 ± 11.1^{o}	54.12 ± 10.5^{o}	77.52 ± 04.1^{o}	64.82 ± 13.6^{o}
RC + E2	E2 (pg/l)	22.36 ± 10.5^{ns}	**233.2 ± 21.1****	**716.89 ± 69.5****	**711.3 ± 55.3****
	P4 (ng/dl)	03.63 ± 06.2^{ns}	**32.01 ± 06.17***	**31.5 ± 11.13***	07.22 ± 03.1
	Prot (g/l)	61.55 ± 09.5^{ns}	**115.3 ± 09.1***	**196.89 ± 15.3***	**181.8 ± 12.1***
	Cho (mg/dl)	71.13 ± 11.3^{ns}	70.71 ± 13.5	69.97 ± 11.2^{ns}	69.11 ± 19.4^{ns}
RC + BC	E2 (pg/l)	31.32 ± 09.2^{ns}	52.55 ± 12.2^{ns}	62.18 ± 10.16^{ns}	61.51 ± 20.3^{ns}
	P4 (mg/dl)	03.55 ± 05.1^{ns}	06.25 ± 04.2^{ns}	11.33 ± 3.36^{ns}	05.32 ± 07.2^{ns}
	Prot (g/l)	71.23 ± 09.3^{ns}	82.14 ± 11.5^{ns}	**169.6 ± 32.1***	**155.6 ± 13.3***
	Cho (mg/dl)	61.73 ± 06.2^{ns}	61.78 ± 05.5^{ns}	68.73 ± 16.1^{ns}	63.67 ± 06.1^{ns}
RC + CP	E2 (pg/l)	25.05 ± 11.1^{ns}	36.65 ± 08.1^{ns}	47.44 ± 25.2^{ns}	50.16 ± 11.2^{ns}
	P4 (ng/l)	04.21 ± 22.3^{ns}	03.03 ± 05.4^{ns}	06.12 ± 10.3^{ns}	07.19 ± 09.3^{ns}
	Prot (g/l)	63.45 ± 23.4^{ns}	99.43 ± 15.1^{ns}	**195.3 ± 11.1***	**191.5 ± 12.2***
	Cho (mg/dl)	51.87 ± 18.3^{ns}	87.15 ± 13.3^{ns}	89.06 ± 14.5^{ns}	90.23 ± 11.6^{ns}

Values are expressed as means (M) ± standard error of the mean (SEM); n = 4. 0: non-significant difference between di-estrus and estrus stages of uncastrated rats; 1 and 2: significant and highly significant difference between di-estrus and estrus stages of uncastrated rats. o: non-significant difference compared to uncastrated rats in the control lot; a and b: significant and highly significant difference compared to uncastrated rats in the control lot. ns: non-significant difference compared to castrated rats in the control group. *: ($p < 0.05$) and **: ($p < 0.01$): significant and highly significant difference compared to castrated rats of the control lot. E2: 17β estradiol; P4: progesterone; ED: distilled water; BC: *Buchholzia coriacea*; CP: *Cogniauxia podolaena*; Prot: protein; Chol: total cholesterol; RnC: non-castrated rats; RC: castrated rats. In bold are significant values.

However, administration of 17β-estradiol to castrated rats resulted in a significant increase in estradiol and progesterone levels compared to control castrated rats ($p < 0.05$).

3.3.2. Total Cholesterol and Protein Levels

The same **Table 3** shows that the administration of the aqueous extract of BC or CP at 600 mg/kg in castrated rats did not cause any change in total cholesterol level ($p > 0.05$), but each extract caused a significant increase in protein level ($p < 0.05$) as with the rats treated with 17β-estradiol, the reference product.

4. Discussion

The present study showed than aqueous extract of the leaves of *Buchholzia coriacea* (BC) Engl. or *Cogniauxia podolaena* (CP) Baill. administered at a dose of 600 mg/kg for one week in castrate d rats did not induce uterotrophic effects, nor did it cause significant variations in the level of sex hormones. However, a significant increase in blood protein levels was observed with both plant extracts.

Ovariectomy of the rats caused a significant increase live weight of the rats compared to uncastrated control rats during the trial period. Administration of 17β-estradiol (E2) to castrated rats significantly decreased this weight compared to control castrated rats that received distilled water. This result is in agreement with those of [23] who reported that, when ovariectomizing female rats, the loss of estrogen production due to the absence of the ovaries caused overfeeding and led to rapid weight gain. [24] reported that in castrated rats, 17β-estradiol facilitates lipolysis and causes weight loss; thus estrogen deprivation leads to obesity and estrogen therapy counteracts this, exerting anti-lipogenetic effects. In this study, 17β-estradiol, by reducing the weight of castrated rats, allowed some restoration of ovarian function after oophorectomy.

However, administration of the aqueous extracts of BC and CP to castrated rats did not cause a significant change in body weight of the castrated rats compared to control rats that received distilled water. The lack of effect of both extracts on body weight in ovariectomized rats suggests that both extracts did not bind to estrogen receptors as with 17β estradiol. Indeed according to [25] [26] [27], 17β estradiol, estrogen and phytoestrogens bind to estrogen receptors, they mimic some of their effects by interaction with ERα and Erβ receptors, even in the absence of the ovaries. Noting that in whole uncastrated or cycled rats, *B. coriacea* caused the decrease in weight and *C. podolaena* caused the increase [12]. These observations suggest that the effect of both extracts on rat weight requires the presence of ovaries, whereas the effect of 17β estradiol on rat weight did not require the presence of ovaries. These extracts are assumed not to have the same mechanism of action as the reference molecule 17β-estradiol, which implies that they would not contain plant hormones or phytohormones.

In addition, administration of the aqueous leaf extract of both plants did not cause a change in the fresh to dry weight ratio of the uterus, an indicator of the level of estrogenic impregnation of the uterus, whereas 17β-estradiol increased

it. The determination of estradiol (E2) and progesterone (P4) levels in this study showed that in uncastrated or cycled control rats, the level of each sex hormone is higher at the estrus stage and lower at the di-estrus stage. This result is in agreement with those reported by [28] and [29] who indicated that in mammals, maximum levels of estradiol and progesterone are observed at the end of proestrus and the beginning of estrus, and minimum levels at di-estrus. Indeed, during the ovulatory phase in women, estradiol levels vary considerably between 30 and 400 pg/ml and progesterone levels between 2 and 25 ng/ml [30] [31]. These values are comparable to the large variations in E2 or P4 levels observed in this study during the estrus phase in the same batch of rats. After ovariectomy, E2 and P4 levels dropped significantly in all rats during the assay period. This indicates that the sexual cycle of castrated rats was effectively blocked.

Administration of *B. coriacea* and *C. podolaena* did not cause significant changes in sex hormone levels (estrogen and progesterone) in castrated rats, whereas 17β-estradiol did. This observation corroborates the hypothesis of the absence of plant hormones or phytohormones in the two extracts studied. Indeed [32] reported that the administration of 17β estradiol, a natural hormone at physiological doses in ovariectomized mice increased the number of estrogen receptors, *i.e.*, the increase in circulating estrogen levels. In addition, both extracts showed no effect on uterine protein or cholesterol levels in castrated rats, however rats treated with 17β-estradiol showed an increase in uterine protein levels and a decrease in uterine total cholesterol levels. According to [15] the increase in uterine protein by estradiol or phytohormones is explained by uterine cell proliferation and the decrease in uterine cholesterol is explained by its use in the process of steroidogenesis. We therefore believe that these extracts would not contain the plant hormones, as they do not provide hormone supplementation, but may be use from hormone phytomodulators.

Blood protein levels increased significantly in rats treated with each plant extract, as with 17β estradiol. This assumes that while uterine protein levels depend on ovarian function, blood protein levels appear to be independent of this function. This study opens up the prospect of deepening the mechanisms of action of phytomodulating plants of hormones and biochemical parameters.

5. Conclusion

The aqueous extracts of the two plants did not show any uterotrophic and hormonal effects in the castrated rat; their estrogenic and progesteronic effects observed in the whole or cycled rat would be ovary-dependent, so they would not contain phytohormones. Both plants could be used as hormone phytomodulators, but their use by traditional medicine in the treatment of hormone deficiencies is not justified.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Adjanohoun, E.J., Ahyi, A.M., Aké Assi, L., Baniakina, J., Chibon, P., Cusset, G., Doulou, V., Enzanza, A., Eymé, J., Goudoté, E., Keita, A., Mbemba, C., Mollet, J., Moutsamboté, J.M., Mpati, J. and Sita, P. (1988) Traditional Medicine and Pharmacopoeia: Contribution to Ethnobotanical and Floristic Studies in the People's Republic of Congo. Agency for Cultural and Technical Cooperation, Paris, 606.

[2] Nkounkou-Loupangou, C., Binimbi-Massengo, A., Ouamba, J.M., Abena, A. and Diatewa, M. (2005) Inventories of Medicinal Plants Used in the Treatment of Infertility in Brazzaville. *Phytotherapy*, **6**, 252-259. https://doi.org/10.1007/s10298-005-0117-7

[3] Bouquet, A. (1969) Fetishists and Traditional Medicine in Congo. O.R.S.T.O.M., Brazzaville, No. 36, 282.

[4] Badila, C. (2003) Contribution to the Chemical and Biological Studies of a Plant Drug with Antidiabetic Effect: The Aqueous Extract of *Cogniauxia podolaena* Baillon Leaves. Single Doctoral Thesis, Marien Ngouabi University, Brazzaville, 134.

[5] Ezeja, M.I., Ezeigbo, I.I. and Madubuike, K.G. (2011) Analgesic Activity of the Methanolic Seed Extract of *Buchholzia coriacea*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2**, 187-193.

[6] Ahombo, G., Ampa, R., Diatewa, M., Mpati, J., Abena, A.A. and Ouamba, J.M. (2012) Investigating on Related Diabetes Therapeutic Plants Used in Traditional Medicine at Brazzaville. *Journal of Medicinal Plants Research*, **6**, 630-639.

[7] Banzouzi, J.T., Soh, P.N., Mbatchi, B., Cavé, A., Ramos, S., Retailleau, P., Berry, A., Benoit-Vical, F. and Rakotonandrasana, O. (2008) *In Vitro* Antiplasmodial Activity of 18 Plants Used in Congo Brazzaville Traditional Medicine. *Planta Medica*, **74**, 1453-1456. https://doi.org/10.1055/s-2008-1081341

[8] Makambila-Koubemba, M.C., Mbatchi, B. and Ardid, D. (2011) Pharmacological Studies of Ten Medicinal Plants Used for Analgesic Purposes in Congo Brazzaville. *International Journal of Pharmacology*, **7**, 608-615. https://doi.org/10.3923/ijp.2011.608.615

[9] Epa, C. (2015) Anti-Inflammatory and Wound Healing Effects of Aqueous and Ethanolic Extracts of *Buchholzia coriacea* Engl. (Capparidaceae), in Mice and Wistar Rats. Single Doctoral Thesis, University Marien Ngouabi, Brazzaville, 183.

[10] Ezekiel, O.O. and Onyeoziri, N.F. (2009) Preliminary Studies on the Antimicrobials Properties of *Buchholzia coriacea* (Wonderful Kola). *African Journal of Biotechnology*, **8**, 472-474.

[11] Adisa, R.A., Choudhary, M.I. and Olorunsogo, O.O. (2010) Hypoglycemic Activity of *Buchholzia coriacea* (*Capparaceae*) Seeds in Streptozotocin-Induced Diabetic Rats and Mice. *Experimental and Toxicologic Pathology*, **63**, 619-625.

[12] Peneme, B.M.L., Okiemy Andissa, N., Mouanké, J.B., Binimbi Massengo, A., Etou Ossibi, A.W. and Abena, A.A. (2015) Contraceptive Effect of Aqueous Extract of *Cogniauxia podolaena* and, *Buchholzia coriacea* Leaves on Fertility in Wistar Rat. *Africa Science*, **11**, 131-140.

[13] Peneme, B.M.L., Etou Ossibi, A.W., Ondele, R., Nsonde Ntandou, G.F., Elion Itou, R.D., Akassa, H. and Abena, A.A. (2018) Effects on Rat Reproductive Parameters of Two Presumed Contraceptive Plants and Their Anti-Oxidant Activities. *International Journal of Multidisciplinary and Current Research*, **6**, 1305-1312.

[14] Bachman, S., Hellwig, J., Jackh, R. and Christian, M.S. (1998) Uterotrophic Assay of Two Concentrations of Migrates of 23 Polystyrenes Administered Orally (by Gavage) to Immature Female Wistar Rats. *Drug and Chemical Toxicology*, **21**, 1-30. https://doi.org/10.3109/01480549809007402

[15] Benie, T., Duval, J. and Thieulant, M.L. (2003) Effects of Some Traditional Plant

Extracts on Rat Oestrous Cycle Compared with Clomid. *Phytotherapy Research*, **17**, 748-755. https://doi.org/10.1002/ptr.1206

[16] Bayala, B., Tamboura, H., Pellicer, M.T.R., Zongo, D., Traoré, A., Ouedraogo, L., Malpaux, B. and Sawadogo, L. (2006) Estrogenic Effects of Aqueous Macerated Leaves of *Holarrhena floribunda* (G. Don) Dur in Ovariectomized Rats. *Biotechnology, Agronomy, Society and Environment*, **10**, 39-50

[17] Munavva, A.S., Nor Azizan, A., Khan, A.A., Aidiahmad, D. and Samshia, D. (2007) Uterotrophy, Fetotoxic and Abortifacient Effect of a Malasian Variety of *Plumbago rosea* L. on Isolate Rat Uterus and Pregnant Price. *Pakistan Journal of Biological Sciences*, **10**, 763-767. https://doi.org/10.3923/pjbs.2007.763.767

[18] Raj, A., Singh, A., Sharma, A., Singh, N., Kumar, P. and Bhatia, V. (2011) Antifertility Activity of Medicinal Plants on Reproductive System of Female Rat. *International Journal of Bio-Engineering Sciences and Technology*, **2**, 44-50.

[19] Boly, H., Peneme, B.M.L., Sawadogo, L., Sulon, J., Beckers, J.F. and Leroy, P.L. (2000) Dose-Response Effect of Gonadotropin (PMSG) on the Reproduction of Djalonké Ewes of Mossi Variety. *Tropicultura*, **18**, 126-129.

[20] Blanchard, S. (2006) Luteal Insufficiency in Domestic Females and the Female. Doctoral Thesis in Veterinary Medicine, Faculty of Medicine of Créteil, Créteil, 130.

[21] Allain, C.C. (1974) Enzymatic Determination of Total Cholesterol in Serum. *Clinical Chemistry*, **20**, 470-475. https://doi.org/10.1093/clinchem/20.4.470

[22] Etou Ossibi, A.W. (2010) Cardiovascular and Antioxidant Effects of Aqueous and Hydro-Ethanolic Extracts of *Lippia multiflora* Moldenke (*Verbenaceae*). Single Doctoral Thesis, University Marien Ngouabi, Brazzaville, 175.

[23] Wade, G.N. and Heller, H.W. (1993) Tamoxifen Mimics the Effects of Estradiol on Food Intake, Body Weight, and Body Composition in Rats. *The American Journal of Physiology—Regulatory, Integrative and Comparative Physiology*, **264**, 12-23. https://doi.org/10.1152/ajpregu.1993.264.6.R1219

[24] Bringer, J., Raingeard, I. and Brun, J.F. (2002) Weight, Nutrition, Exercise and Premenopause. Extract from Updates in Medical Gynecology. Volume 2002, National College of French Gynecologists and Obstetricians, Montpellier, 18 p.

[25] Savouret, J.F. (2005) Phytoestrogens and Their Perspectives. 7(4) Inserm UMR-S-530, University Paris 5 UFR Biomédicale, Paris, 20.

[26] Abdoulaye, B. (2015) Modulatory Effects of 17-beta Estradiol on Dopamine D2 Receptors: Consequences on Body Weight, Food, Water, Alcohol and Sugar Intake. 40. https://www.researchgate.net/publication/280115033

[27] Fenichel, P., Brucker, D.F. and Chevalier, N. (2016) Endocrine Disruptors-Reproduction and Hormone-Dependent Cancers. *La presse médicale*, **45**, 63-72. https://doi.org/10.1016/j.lpm.2015.10.017

[28] Thibault, C. and Levasseur, M.C. (2001) La reproduction chez les mammifères et l'homme. Ellipses: INRA éditions, Paris, 928.

[29] Gayrard (2007) Physiology of Mammalian Reproduction. Toulouse Veterinary School, Toulouse, 198.

[30] Marieb, E.N. and Hoehn, K. (2015) Anatomie et physiologie humaines. Nouveaux horizons. ARS, Paris, 1308 p.

[31] Cypress Diagnostics (2016) Elisa Test for Estradiol and Progesterone. Reference HE503, 96 Tests/Kits, 8.

[32] Chalmey, C. (2013) Neonatal Exposure to Estrogens: Effects on Their Metabolism, Ovarian Development and Reproductive Function in the Rat. PhD Thesis, Mention Biology, University of Rennes 1, Rennes, 293.