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Anti-androgenic, anti-oestrogenic and antioxidant activities of aqueous extract of *Laportea ovalifolia* on adults rats.

Sylvain Nguedia Njina[1], Richard Simo Tagne[1,2], Bruno Phelix Telefo[1*], Francois Ngoufack Zambou[1], Didiane Mefokou Yemele1, Felicite Tchouanguep Mbiapo.[1]

***Corresponding author:**

Bruno Phelix Telefo

[1] Laboratory of Biochemistry of Medicinal Plants, Food and Nutritional Sciences (LABPMAN), Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.
[2] Department of Biomedical Sciences, University of Ngaoundere, P.O. Box 454, Ngaoundere,

Abstract

Cancer is one of the most life-threatening diseases in which deregulating proliferation of abnormal cells invades and disrupts surrounding tissues. It constitutes serious public health problems in both developed and developing countries.

Objective: To evaluate the anti-androgenic, anti-estrogenic and antioxidant activities of *Laportea ovalifolia* (*L. ovalifolia*) in order to contribute to the search and the valorization of medicinal plants which could reduce mortality related to prostate cancer.

Methods: The evaluation of the anti-androgenic activity were carried out on castrated male rats receiving simultaneous daily administration of testosterone and different doses of aqueous extract of *L. Ovalifolia* during a period of 10 days. That of the anti-estrogenic activity was carried out on mature ovariectomized female rats receiving for a week simultaneous daily administration of estradiol and different doses of plant extract. The evaluation of the *in vivo* antioxidant activity of *L. Ovalifolia* aqueous extract was carried on adult male rats receiving simultaneous daily administration of naphthalene and different doses of extract, for 15 days. For its *in vitro* antioxidant activity, the amounts of phenolic compounds in plant extracts were determined as well as the total flavonoid contents of the crude extracts. Also, the DPPH scavenging activity of the plant extract was determined as well as its reducing power.

Results: As compare to the 0 mg/kg testosterone primed castrated rat, those treated with the various dose of the plant extract presented either a significant decrease in weights of all their reproductive tissues ($P<0.01$ - $P<0.001$) or a significant increase ($P<0.001$) in their serum level of testosterone. For all the plant extract treated ovariectomized rats, similar trends were observed for the relative uteri weight ($P<0.01$) and that of the serum level of estradiol ($P<0.001$). Plant extract contains 13.33±0.1 mg GAE/g and 05.27±0.17 mg CATE/g of phenolic and flavonoids compounds respectively and exhibits DPPH radical scavenging ability as well as ferric-reducing antioxidant power. Relatively to animals treated at 0 mg/kg, the various doses of the plant extract significantly increased ($P<0.05$ - $P<0.001$) the activity of catalase (in liver, lungs and the serum), SOD (in liver and heart) and peroxidase (in liver, heart, serum and lungs). It also significantly reduces ($P<0.001$) the level of nitric oxide in the liver, heart, lungs, kidneys and serum.

Conclusions: Globally, these results denote the anti-androgenic, anti-estrogenic and antioxidant potential of *L. ovalifolia*.

Keywords: *Laportea ovalifolia,* prostate cancer, anti-androgenic activity, anti-estrogenic, antioxidant activity.

Introduction

Despite efforts made in the fight against prostate cancer, the death rate related to this disease is up to now growing-up in the world. It is characterized by proliferation of abnormal cells that invade and disrupt surrounding tissues [1].

Living organisms use oxidative process for the production of energy usable to maintain their integrities and this oxidative process is one of the most important routes for producing free radicals that plays an important role in degenerative diseases including cancer [2, 3].

Huggins and Hodges [4] showed the direct relationship between androgens and prostate cancer, they have demonstrate that prostate cancer was initiated by androgenic hormones, and its evolution and dissemination could be inhibited by elimination of androgens through castration, the administration of anti-androgens or the use of LHRH analogues. These therapies have considerably ameliorated the management of this form of cancer with the development of new drug with anti-androgenic potential such as flutamide and bicalutamide. Despite the variety of drugs used in this therapy, treatment is not completely effective and almost all treated patients eventually relapse. This relapse is also associated with progression of the disease to an androgen-independent state, for which there is no known treatment.

Estrogens have been used as potential agents in the development and progression of prostate cancer [5] and it is becoming clear that a finely tuned balance between the effects mediated by androgen (AR) and estrogens receptors (ERα, and ERβ), is required for the maintenance of prostate health. The action of estrogens is complex, having both adverse and beneficial roles via ERα and ERβ respectively [6].

Medicinal plants have shown beneficial properties in the treatment of various types of cancers due to their content in secondary metabolites. The anti-estrogenic potential of some natural or synthetic compounds are becoming of great interest since the discovery of the implication estrogen receptors in prostate malignancy. Indeed, Tagne *et al.* [7] have shown that the aqueous extract of *Eremomastax speciosa* contains compounds with anti-androgenic, anti-estrogenic and antioxidant properties. They have also shown that methanolic extract of this plant contain compounds presenting antiproliferatives properties on certain cancer cell types [8]. In addition, Raloxifene (a non steroid anti-estrogen) was shown to induce apoptosis of both androgen dependent and independent prostate cancer cell lines *in vitro* [9].

In view to contributing to the search and the valorization of medicinal plants with anti-androgenic, anti-estrogenic and antioxidant potential, *Laportea ovalifolia* (*L. ovalifolia*), a medicinal plant of the Urticaceae family which is used in the traditional system of medicine in Cameroon for the treatment of infertility, bacterial diseases and benign prostatic hyperplasia were investigated in the present study.

Materials and methods

Plant and extract preparation

The fresh leaves of L. ovalifolia were collected in February 2013 in the locality of Dschang (Cameroon) and have been identified in the National Herbarium of Cameroon under voucher specimen code 49533/HNC. They were dried at room temperature and ground in a mortar into powder. Ten grams (10g) of the powder was submerged into 100 mL of boiling distilled water and the setup was kept boiling for 30 minutes. After cooling, the extract was filtered before being dried in a ventilated oven at 45 C.The powder extract was stored in the refrigerator at - 20 C for further used. The extract yield was 5%.

Animals

Anti-androgenic and anti-estrogenic assay were conducted on 42-45 days old albino Wistar rats of both sex, weighing 80 – 150 g while the in vivo antioxidant test necessitated 60 days old male albino Wistar rats, weighing 150–200 g. The rats were bred in the animal house of the Biochemistry Department (University of Dschang, Cameroon), housed under natural conditions of light (12 h cycles) and temperature (22 ± 2 C) and fed with standard laboratory diet and tap water ad libitum. The care and handling of the animals as well as the experiments were conducted in accordance with the international accepted standard guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [10].

Evaluation of anti-androgenic activity

The test was carried out on castrated male rats. Ten days after castration, rats were randomized and separated into five groups, each of which consisted of six animals. Groups I to V received simultaneously testosterone (0.4 mg/kg) and flutamide (3 mg/kg) or increasing doses (0, 100, 400, 800 mg/kg) of plant extract. The treatment of animals was carried out over a period of ten days. Twenty-four hours after the last dose, animals were sacrificed under chloroform anesthesia and their blood extracted from the heart. The serum was separated and used for serum biochemistry. Reproductive tissue like ventral prostate gland, levator ani bulbocavernosus (LABC) muscle, cowper's glands (CG), the glands penis (GP) were excised, blotted free of blood and weighed and serum testosterone level was determined using ELISA testosterone test kit manufacture by Fortress diagnostics, catalog number BXE0862A.

Evaluation of anti-estrogenic activity

Experiments were conducted on mature ovariectomized female albino Wistar rats aged 42 days. One week after ovariectomy, rats were distributed in five groups with six animals in each group. Group I to V received daily, subcutaneously or orally, estradiol (0.05 µg/kg) and raloxifene (0.1 mg/kg) or the various doses of the aqueous plant extracts (0, 100, 400 and 800 mg/kg). Twenty-four hours after the last dose, animals were sacrificed under chloroform anesthesia and their blood extracted from the heart. The serum was separated and used for serum biochemistry. Uteri were excised and its weights were recorded. Serum level of estradiol

was determined using the estradiol enzyme immunoassay test kit manufactured by General Biologicals corporation, catalog number 4S00071.

Evaluation of *in vitro* antioxidant activities

Total phenolic compound analysis

The amounts of phenolics compounds in plant extracts were determined with Folin-Ciocalteu reagent using the method of Spano and Wrolstad [11], as modified by Lister and Wilson [12]. To 50 ml of each sample (3 replicates), 2.5 ml of 10% dilution of Folin-Ciocalteu reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and the resulting mixture was incubated at 45 ˚C for 15 min. Gallic acid was used as the standard for the calibration curve. The absorbance of all samples was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Total flavonoid content determination

Total flavonoid contents of the crude extracts were determined according to the method of Lamaison and Carnet [13]. Aliquots of 1.5 mL of extract were added to an equal volume of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 mL methanol) solution. The mixture was vigorously shaken, and the absorbance was read after 10 min of incubation at 430 nm. Catechin was used as the standard for the calibration curve. The total flavonoid content was calibrated using the linear equation based on the calibration curve. The total flavonoid content was expressed in milligram of catechin equivalent/gram of dry weight. The dry weight indicated was the sample dry weight.

DPPH Free radical scavenging assay

The DPPH scavenging activity of the plant extract was determined according to the method reported by Gyamfi [14]. The samples (25, 50, 100 and 200 µg/ml), was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, reading the absorbance at 517 nm. Vitamin C was used as control. The percent inhibition was calculated from the following equation:

% Inhibition = [Absorbance of control -Absorbance of test sample/Absorbance of control] * 100.

Reducing power measurement

The reducing power of the crude extracts were determined according to the method of Gow*et al.* [15]. The samples (25, 50, 100 and 200 µg/ml) were each mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 C for 20 min before an equal volume of 1% TCA was added, and then centrifuged at 5,000 g for 10 min. The supernatant of the solution was mixed with distilled water and 0.1% $FeCl_3$ on a ratio of 1: 1: 2 respectively and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated an increase in reducing power.

Evaluation of the *in vivo* antioxidant activities

Experiments were performed on six groups of six rats. The 1st to 5th groups were given simultaneously naphthalene solution (110 mg/kg, p.o.) and vitamin C (3 mg/kg) or plant extract at doses of 0, 100, 400 and 800 mg/kg, p.o. and the last group was administered an equal volume of the vehicle (tournesol oil) orally. All treatment was performed within a period of 15 days. Twenty-four hours after the last treatment, all animals were sacrificed under chloroform anaesthesia and their blood samples were extracted from the heart. Afterwards, serum was quickly separated by centrifugation for 15 min at 3000 g, and kept at -80 Cº until used for serum biochemical assays. The liver, heart, lungs, and kidneys were also immediately removed and were washed in chilled saline and kept at -80 Cº until the used for tissues biochemitry. Superoxide dismutase and catalase were determined in this study by the method of Pavlovic *et al.* [16] peroxidase was evaluated by using the method of Habbu *et al.* [17] and the nitric oxide by the method using the Griess reagent system

Statistical analysis

The data obtained from biological assays were analysed by the one way analysis of variance (ANOVA) test and expressed as mean ± SEM. The Fisher's exact test was used for the comparison of means.

Results

Anti-androgenic activity of aqueous plant extract

Variations of the weight of androgen-dependent glands according to various treatments are presented in **Figure 1**. A significant reduction ($P<0.01$ - $P<0.001$) of weights of all reproductive tissues was observed with flutamide treated castrated rat as well as those receiving the plant extract when compared with that of the negative control group (0 mg/kg).

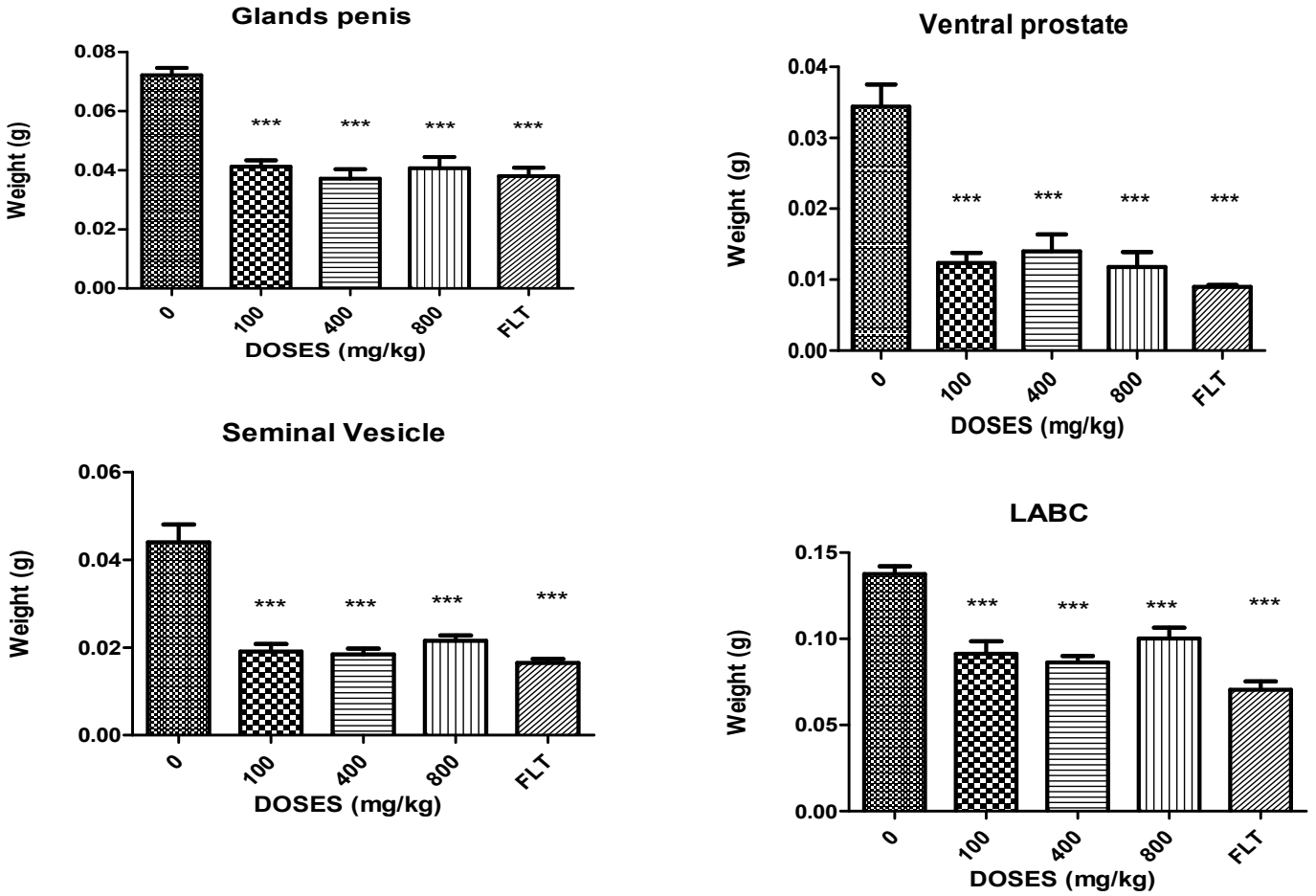

Figure 1. Weights of androgen depending organs and tissues according to the various groups of treatment. Each value represents means ±S.E.M. of 6 rats. *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg). FTL : flutamide.

Effect of the plant extract on the Serum level of testosterone

Serum levels of testosterone in all treated animals are presented in **Figure 2**. It is noticed that flutamide treated castrated rat as well as those receiving the plant extract at doses of 100 and 400 mg/kg presented a significant increased (P<0.001) in the serum level of testosterone when compared with that of the negative control group (0 mg/kg).

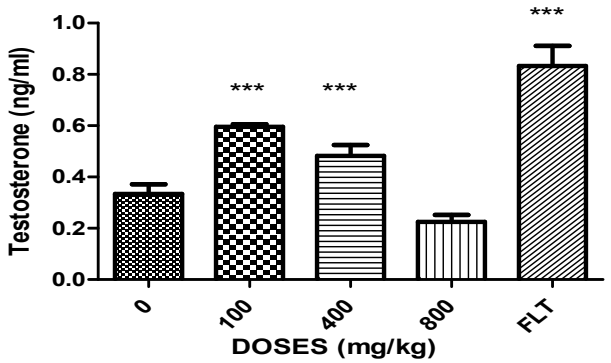

Figure 2. Serum level of testosterone according to the various groups of treatment. Each value represents means ±S.E.M. of 6 rats. *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg). FTL : flutamide

Anti-estrogenic activity of aqueous plant extract

As presented in **Figure 3** below, Raloxifene (P<0.001) and the plant extract (P<0.05 - P<0.01) significantly reduced the relative weight of uteri as compared with that of the negative control group (0 mg/kg).

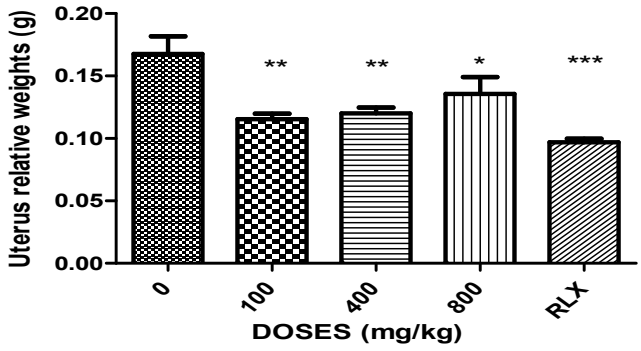

Figure 3. Relative weights of uteri according to the various groups of treatment. Each value represents means ±S.E.M. of 6 rats; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg).RLX : Raloxifene

Effect of the plant extract on the Serum level of estradiol

Variations of serum level of estradiol according to the various groups of treatment are presented in **Figure 4**. The plant extract at doses of 400 and 800 mg/kg (P<0.05 to P<0.001) as well as raloxifene (P<0.001) have significantly increased the serum level of estradiol when compared with dose negative control group (0 mg/kg).

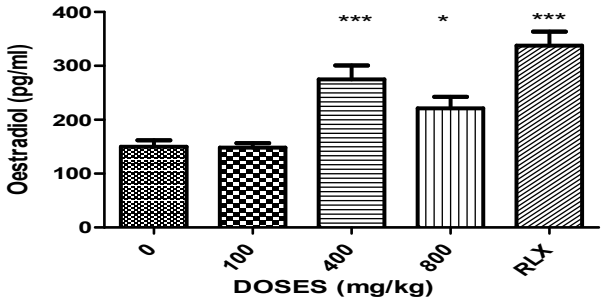

Figure 4. Variation of serum level of estradiol according to the various groups of treatment. Each value represents means ±S.E.M. of 6 rats; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg). RLX : Raloxifene.

Total phenolics and total flavonoids content of aqueous plant extract

The total phenolic content, expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g), and total flavonoid content expressed as milligrams of catechine equivalent per gram of dry weight (mg CATE/g) were 13.33 ±0.1 and 05.27±0.17 respectively.

DPPH radical scavenging activity

The dose-response curve of DPPH radical scavenging activity of the extracts of *L. Ovalifolia* compared with that of vitamin C (**figure 5**) show that there is a significant difference between the scavenging activity of the plant extract and that of vitamin C at concentrations of 25 µg/ml (P<0.001) and 100 µg/ml (P<0.05).

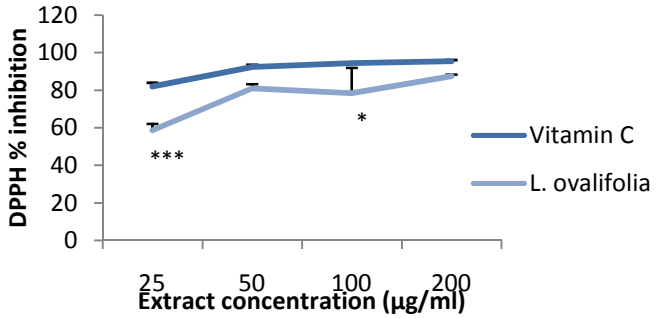

Figure 5: DPPH radical scavenging activities of extracts. Each value represents means ±S.E.M. of three repetitions; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (Vitamin C).

Ferric reducing antioxidant power

Figure 6 represents the dose-response curve of the ferric-reducing antioxidant power (FRAP) of the extract of *L. Ovalifolia* as compared to that of vitamin C. Data show that there is a significant difference between the ferric-reducing antioxidant power (FRAP) of the plant extract and that of vitamin C at concentrations of 50 µg/ml (P<0.05), 100 µg/ml (P<0.01) and at 200 µg/ml (P<0.001).

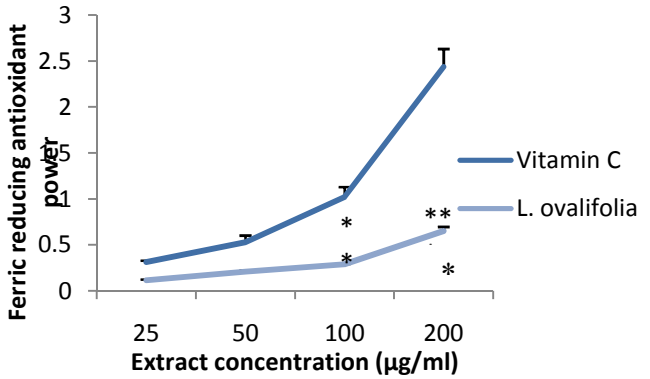

Figure 6: The ferric-reducing antioxidant power (FRAP) of extracts; Each value represents means ±S.E.M. of three repetitions; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (Vitamin C).

Effect of the aqueous extract of plant on the activity of catalase

Data (**Table 1**) revealed that naphthalene (0 mg/kg treated animals) induced a significant reduction in heart and serum activities (P<0.001) of catalase when compared to that of tournesol oil treated animals. However the administration of vitamin C as well as various doses of the plant extract to the naphthalene treated animals significantly increased catalase activites in the heart, kidney, serum (P<0.001) as well as the liver and lungs (P<0.01 to P<0.001).

Table 1 Activity of catalase in liver, heart, lungs, kidneys (UI/mg enzyme) and serum (UI/ml enzyme) according to various groups of treatment.

Doses	*Liver*	*Heart*	*Lungs*	*Kidneys*	*Serum*
Tournesol oil	528.11±133.20	53.16±7.14 ***	43.41±9.21	160.66±14.74	239.91±5.46 ***
0 mg/kg	442.80±94.75	14.91±3.29	50.67±11.49	111.28±24.94	133.11±13.89
Vitamin C	640.81±117.99	39.05±9.16 ***	46.12±9.34	283.1±51.06 ***	272.31±9.64 ***
100 mg/kg	769.77±191 **	9.13±1.60	69.91±16.21	153.54±20.15	218.85±8.55 ***
400 mg/kg	644.53±139.24	5.88±0.51 *	130.98±21.66 ***	153.94±37.66	246.12±46.77 ***
800 mg/kg	652.19±110.63	18.85±5.45	103.12±20.47 ***	149.90±35.60	201.84±23.64 **

Each value represents means ±S.E.M. of 6 rats; *: P<0.05; **: P<0.01; ***: P<0.001 significantly different compared to control (0 mg/kg).

Effect of the aqueous extract of plant on the activity of superoxide dismutase (SOD)

Superoxide dismutase activity in tissues and serum (**Table 2**) revealed that naphthalene (0 mg/kg treated animals) have induced a significant reduction of the SOD activities in liver and kidneys (P<0.05, P<0.001) when compared to that of tournesol oil treated animals. The administration of vitamin C as well as plant extract to the naphthalene treated animals significantly increase this activity in the liver and the heart (P<0.05 - P<0.001).

Table 2: Activity of superoxide dismutase in the liver, heart, lungs, kidneys (UI/mg enzyme) and serum (UI/ml enzyme) according to various groups of treatment.

Doses	*Liver*	*Heart*	*Lungs*	*Kidneys*	*Serum*
Tournesol oil	336.61±28.26 ***	39.44±5.91	43.90±6.60	100.09±7.2 *	43.44±1.57
0 mg/kg	176.29±35.41	32.04±6.98	41.35±7.65	77.47±16.87	41.62±3.53
Vitamin C	288.40±35.50 *	37.85±7.66	37.24±6.85	58.53±7.47	43.52±2.51
100 mg/kg	279.35±44.25 *	53.13±6.37 ***	35.25±6.77	92.68±15.70	41.37±3.94
400 mg/kg	370.32±92.48 ***	40.06±5.22	45.67±6.57	92.26±12.98	43.61±5.15
800 mg/kg	132.21±26.78	47.83±2.67 **	56.12±13.70	89.64±16.66	43.31±1.76

Each value represents means ±S.E.M. of 6 rats; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg).

Effect of the aqueous extract of plant on the activity of peroxidise

Data present in table 3 revealed that the administration of naphthalene (0 mg/kg treated animals) have induced a significant reduction of the peroxidase activity in kidneys and in the heart (P<0.05 - P<0.001) when compared to that of tournesol oil treated animals, whereas the administration of vitamin C significantly increase this activity in kidneys and in the heart (P<0.01 - P<0.001) as well as the plant extract in lungs, serum, heart and the liver (P<0.05 to P<0.001).

Table 3: Activity of peroxidase in the liver, heart, lungs, kidneys (UI/mg enzyme) and serum (UI/ml enzyme) according to various groups of treatment.

Doses	*Liver*	*Heart*	*Kidneys*	*Serum*	*Lungs*
Tournesol oil	381.40±79.17	29.08±5.78 *	56.39±10.32 ***	0.47±0.04	39.12±5.42
0 mg/kg	279.75±41.27	15.37±3.31	20.46±2.07	0.49±0.08	37.43±3.43
Vitamin C	354.47±55.76	32.63±8.57 **	49.04±10.13 ***	0.52±0.06	39.85±7.78
100 mg/kg	318.96±31.19	22.77±6.46	34.91±7.44	0.61±0.10	78.87±13.75 ***
400 mg/kg	392.68±77.43 *	27.49±5.32 *	31.89±5.75	0.65±0.13 *	62.54±8.45 **
800 mg/kg	406.93±42.69 *	25.54±4.70	30.42±2.48	0.43±0.03	50.83±8.5 *

Each value represents means ±S.E.M. of 6 rats; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg).

Effect of the aqueous extract of plant on the level of nitric oxide

Data illustrated in **table 4** revealed that naphthalene (0 mg/kg treated animals) have significantly increase the level of nitric oxide in the liver and heart (P<0.01 - P<0.001) when compared to that of tournesol oil treated animals and the administration of vitamin C significantly decrease this level of nitric oxide in serum and most of the tissues (P<0.05 to P<0.001) as well as plant extract in the serum and almost all tiss[[ues (P<0.05 to P<0.001).

Table 4: Level of nitric oxide in the liver, heart, lungs,kidneys (ng/mg) and serum (ng/ml) according to various groups of treatment.

Doses	*Liver*	*Heart*	*Lungs*	*Kidneys*	*Serum*
Tournesol oil	8.30±1.62 ***	3.51±0.54 **	20.77±1.17	10.67±0.48	2.66±0.06
0 mg/kg	23.98±1.19	5.30±1.10	21.47±2.62	11.22±2.84	3.01±0.70
Vitamin C	12.1±3.88 ***	3.22±0.65 ***	18.75±0.40	7.35±0.94 **	2.03±0.55 *
100 mg/kg	17.85±1.38 **	2.69±0.53 ***	10.5±2.3 ***	3.92±0.58 ***	3.20±0.50
400 mg/kg	13.34±2.56 ***	2.94±0.25 ***	22.14±0.3	6.38±0.99 **	4.28±0.74 **
800 mg/kg	13.72±0.71 ***	4.19±0.36 *	17.42±2.05**	6.54±1.49 **	2.04±0.29 *

Each value represents means ±S.E.M. of 6 rats; *: P<0.05; ** : P<0.01 ; *** : P<0.001 significantly different compared to control (0 mg/kg).

Discussion

It has been shown that Prostate cancer depends mainly for androgens activity. Reduction or inhibition of these androgens activities remain the principal therapeutically means used in the treatment and management of this form of cancer. A significant

reduction ($P<0.01$ - $P<0.001$) of weights of all reproductive tissues was observed with flutamide treated castrated rat as well as those receiving the plant extract when compared with that of the negative control group (0 mg/kg). Since androgens are involved in cell proliferation, the decrease in the weight of organs and glands may be due to the presence in the extract, of molecules such as anti-androgens that can inhibit the action of testosterone. Anti-androgens bind to androgenic receptors thus preventing their recognition by androgens [18]. This shows that the extract may contain steroid or non steroid anti-androgenenic substances which inhibit the effect of testosterone propionate. These substances may also behave as 5α-reductase inhibitors. Indeed, this enzyme converts in prostatic cells, testosterone to dihydrotestosterone, the principal agonist of androgen receptor [19].

We have demonstrated in this study that the aqueous extract of *L. Ovalifolia* contained 05.27±0.17mg CATE/g dw of flavonoid. Phytoestrogens are classified into isoflavonoids, flavonoids, stilbenes, lignans and others on the basis of their chemical structure [20]. Phytoestrogens are being studied extensively for their potential roles in hormone-sensitive cancers such as prostate, breast and colon cancers [21]. Pihlajamaa *et al.* [22] recently reported that genistein is a tissue-specific androgen receptor modulator. It was noted that genistein had an anti-androgenic effect on testis, prostate and brain in non castrated male mice, whereas androgenic effect was observed on prostate and brain tissues of the castrated mice. Flavonoids contain in this extract could be phytoestrogens.

Leon *et al.* [23] have reported the effect of flutamide on testosterone metabolism and the plasma levels of androgens. Their work revealed that flutamide, a non-steroidal anti-androgen, significantly increased plasma level of testosterone. The significant increase of testosterone level observed at doses of 100 and 400 mg/kg could be due to the presence in the plant extract of anti-androgenic substances that act as flutamide.

Phytoestrogens are biologically active phytochemicals with estrogenic and/or anti-estrogenic effects in the human body [24]. The significant decrease in the relative weight of the uteri observed in animals receiving various doses of extract could be explained by the presence of flavonoids in the aqueous extract of *L. ovalifolia*. It has been established that some phyto-estrogen are flavonoids and can exhibit anti-estrogenic activities [25]. Androgens are essential for the regulation of prostate growth and functions; intra-prostatic metabolism of testosterone to estrogens has been recently proposed to play a vital role in the regulation of prostate gland growth [26]. Estrogenic stimulation through estrogen receptor in a milieu of decreasing androgens contributes significantly to the genesis of benign prostatic hyperplasia, prostate dysplasia, and prostate cancer [27]. Piccolella *et al.* [28] show that synthetic and natural modulators of ER action may exert a protective action against the progression of prostate cancer even in its androgen-independent status. Prostate carcinogenesis suppression by anti-estrogens and selective estrogen receptor modulators (SERMs) is supported by preclinical, clinical, and epidemiological studies [29].

Brigitte *et al.* [30] have shown that raloxifene (a non steroid anti-estrogen) treatment is associated with increased serum estradiol in healthy middle-aged men. The significant increase of estradiol level at 100 and 800 mg/kg could be due to the presence in the plant extract of anti-estrogenic substances that act as raloxifene.

The content in total phenols of this plant extract is 14.2 ± 0.07 mg GAE/g dw. Phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to their redox properties [31]. DPPH radical scavenging ability of this extract demonstrates that the phenols present in the plant extract possess high radical scavenging ability. The beneficial effects of natural antioxidants in fruits and vegetables are partly related to their phenolic contents and this could equally explain the reducing power of our plant extract.

Naphthalene toxicity is highly species, tissue and cell selective [32]. It has been found to cause cataract, haemolytic anemia, and damage of the bronchial epithelial (Clara) cells and proximal tubules of the kidney [33]. Moreover, naphthalene was involved in hepatocyte injury and liver dysfunction [34]. Toxic manifestations of naphthalene have been attributed to oxidative stress caused by the generation of reactive oxygen species (ROS) [35].

Globally, results of the present study show that naphthalene have significantly decrease the activities of the three antioxidants enzymes involves in this study in both organs and serum of naphthalene treated animals in comparison with tournesol oil (vehicle) treated animals; whereas plant extracts and vitamin C have protected animals from the toxic effect of naphthalene in animals treated with both naphthalene and plant extracts and with naphthalene and vitamin C. These beneficial effects exhibited by the plant extract may be explained by the presence of natural antioxidants as demonstrated in this study. *L. ovalifolia* aqueous extract significantly increase the activities of all of the three enzymatic antioxidants studied, suggesting that it could be used to prevent oxidative stress.

Nitric oxide (NO) is itself a reactive nitrogen species (RNS) produced in a variety of cells and its effects on different types of cells have proven to be either protective or toxic, depending on its concentration [36]. An inappropriately high concentration of NO has been implicated in several diseases [37]. Results from the present study show that the plant extract inhibits the production of higher concentration of NO, this may be explained by the presence in the extract of molecules that can act as inhibitors of the synthesis of inducible isoform (iNOS) of nitric oxide synthase responsible for the generation of large and sustained amounts of NO from many cell types.

Conclusion

Globally, our study demonstrated that *L. ovalifolia* possess anti-androgenic, anti-estrogenic and antioxidante activities. These activities of *L. ovalifolia* might at least in part, be related to it contain

in secondary metabolites such as polyphenolic and flavonoids compounds or other classes of secondary metabolite compounds.

Authors' contribution

NN carried out the literature search, the field and laboratory investigations and drafted the manuscript.
ST and MY participated in the literature search and assisted in the laboratory investigations.
NZ and MT revised the manuscript and prepared it for publication.
PT conceived and designed the study and supervised the laboratory investigations.
All authors read and approved the manuscript.

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