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Phenolic compound contents and effect of aqueous extract of *Cordyline fruticosa* leaves on testosterone-induced benign prostatic hyperplasia in male Wistar rats

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Abstract

Aim: Benign prostatic hyperplasia has become a major public health problem. This study aimed to evaluate the phenolic compound contents and the effect of the aqueous extract of *Cordyline fruticosa* leaves on benign prostatic hyperplasia induced in Wistar rats.

Method: The phenolic compound contents were determined using colorimetric methods. To study the effect of the aqueous extract on benign prostatic hyperplasia, one group of male rats served as normal controls, while four groups of male rats received subcutaneous injections of testosterone propionate for 28 days. Among these four groups of male rats, one group of rats received no treatment, while the other three groups received daily oral doses of 200; 400 mg/kg body weight of *Cordyline fruticosa* aqueous extract and 10 mg/kg body weight of finasteride for 28 days. Rats were sacrificed at the end of the study and their blood was collected for biological analyses. Prostates of the rats from the different batches were also weighed at the end of the study.

Results: The aqueous extract of *Cordyline fruticosa* recorded total phenol contents andtotal flavonoidsrespective of92.34 ± 2.87 mg EAG/gof extractAnd15.21 ± 2.59 mg EQ/g of extract. The aqueous extract of *C. fruticosa* at doses of 200 mg/kg bw and 400 mg/kg bw significantly reduced prostate weight in diseased rats. Also, these extracts had positive actions on hematological and biochemical parameters evaluated during benign prostatic hyperplasia.

Conclusion: These results justify the use of *Cordyline fruticosa* in African pharmacopoeia for the treatment of benign prostatic hyperplasia.

Keywords: *Cordyline fruticose*; Phenolic compounds; Benign prostatic hyperplasia; Biochemical parameters; Prostate specific antigen

1. Introduction

During aging, the prostate, a gland of the male genital tract located below the bladder and surrounding the urethra, develops and increases in volume due to excessive cell proliferation in the transition zone [1]. This physiological phenomenon can lead to various pathologies such as hypertension, prostate cancer or benign prostatic hyperplasia (BPH) [2]. BPH, commonly called prostatic adenoma or benign prostatic hyperplasia, is the most common benign tumor in adult men [3]. It can remain asymptomatic or become symptomatic when it causes lower urinary tract symptoms such as dysuria, nocturia, difficulty initiating urination, difficulty emptying the bladder, etc. [4].

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In recent years, several drugs containing testosterone, an androgen hormone produced by the testes in men, have been prescribed to men to help them maintain good health [5]. However, increased serum testosterone levels throughout life can cause several diseases. Gonzales et al. (2012) [4] and Park et al. (2016) [6] reported that this hormone can sometimes cause irregular growth of prostate stromal tissue leading to either prostate cancer or benign prostatic hyperplasia (BPH) [3].

Although the management of BPH requires drug or surgical treatments [3, 7], the increasingly frequent use of medicinal plants, as an alternative for the treatment of this disease, has been mentioned by several authors during their research [8, 9].

Cordyline fruticosais a shrub with a slender trunk that can reach 4 to 5 m in height. The leaves are used to treat sore throats and pain [10], while the roots are used to treat toothaches, laryngitis and mammary gland infections [11]. In Ivory Coast, this plant is commonly used by some populations in decoction to relieve pain caused by benign prostatic hyperplasia.

The objective of this study was to evaluate the phenolic compound contents and the effect of the aqueous extract of Cordyline fruticosa leaves on benign prostatic hyperplasia induced in male Wistar rats by testosterone.

2. Materials and methods

2.1. Plant material

The plant material consists of Cordyline fruticosa leaves (Figure 1). These leaves were harvested in March 2022 in Daloa, a town located in the Center West of Côte d'Ivoire in the Haut-Sassandra region, and authenticated by the plant biology and physiology laboratory of the Jean Lorougnon GUEDE University.

Figure 1 *Cordyline fruticos***a**

2.2. Experimental animals

For this study, male Wistar rats weighing between 251 and 281 g were used. These male rats were raised under standard temperature, nutrition, and atmospheric pressure conditions in the animal facility of the Jean Lorougnon GUEDE University in Daloa. They were fed with food supplied by IVOGRAIN® of Abidjan (COTE D'IVOIRE) and had free access to water.

2.3. Preparation of aqueous extract of Cordyline fruticosa leaves

A fine plant powder was obtained after pulverizing the dried leaves of C. fruticosa. Thus, 100 g of this fine powder was added to 1 L of distilled water and the whole was brought to a boil for 15 minutes. After cooling, the decoction was drained in a square of clean cloth, then filtered twice through hydrophilic cotton and once through Whatman filter paper (3 mm). The filtrate obtained was then slowly dried in a Med Center Venticell oven at 50 ° C for 72 hours. The extract obtained is an aqueous decoction which was stored in sterile and dry bottles for the rest of the manipulations.

2.4. Determination of total phenol content

The determination of total phenols was carried out according to the method described by Wood et al. (2002) [12]. To a volume of 30 µL of extracts were added 2.5 mL of Folin-Ciocalteu reagent diluted 1/10. The mixture obtained was kept for 2 min in the dark at room temperature (27 ± 30 °C) and then 2 mL of sodium carbonate solution at 75 g/L was added. The solution obtained was then incubated at 50 °C for 15 min. The absorbance reading was carried out with a UV-visible spectrophotometer at a wavelength of 760 nm against a blank consisting of 5 mL of Folin-Ciocalteu reagent diluted 1/10 and 4 mL of the sodium carbonate solution at 75 g/L. Gallic acid was used as a reference standard for establishing the calibration curve and for quantifying total phenol contents expressed in mg gallic acid equivalent per gram of extract (mg EAG/g extract).The tests were carried out in triplicate for each sample.

2.5. Determination of total flavonoid content

Total flavonoid content was determined according to the method described by Marinova et al. (2005)[13]. Volumes of 0.75 mL of 5% (m/v) sodium nitrite solution and 0.75 mL of 10% (m/v) aluminum chloride solution were added to 2.5 mL of 1/500 (m/V) extract solution. After 5 min of incubation, the mixture was brought into contact with 5 mL of 1 M sodium hydroxide solution. The resulting volume was adjusted to 25 mL and then shaken vigorously. Absorbance was measured at a wavelength of 510 nm against a blank consisting of 0.75 mL of 5% (w/v) sodium nitrate solution, 0.75 mL of 10% (w/v) aluminum chloride solution, and 5 mL of 1 M sodium hydroxide solution. Quercetin was used as a reference standard for establishing the calibration curve and for quantifying total flavonoid contents expressed as milligrams of quercetin equivalent per gram of extracts (mg EQ/g of extract). The tests were carried out in triplicate for each sample.

2.6. Induction of benign prostatic hyperplasia in male Wistar rats

Testosterone propionate (TP) was used to induce benign prostatic hyperplasia (BPH) in male Wistar rats according to models proposed by Shan et al. (2022) [9] and Chung et al. (2024) [14] with some slight modifications. The testosterone propionate solution was prepared in olive oil used as a carrier agent.

Thus, after one week of acclimatization in the laboratory, 25 male rats of Wistar strain whose weights varied between 251 and 281 g were randomly divided into five (05) groups (N = 5 per group).

Group 1 remained healthy (no diseased and control group). Groups 2, 3, 4 and 5 were made sick by injecting Testosterone propionate (3 mg/kg bw) into individual rats in these groups for 28 days. The experimental protocol was summarized as follows :

- Group 1: Normal group (GN) receiving distilled water orally (0.5 mL/100 g body weight) and olive oil subcutaneously (0.3 mL/100 g body weight) for 28 days.
- Group 2: Group of diseased rats given subcutaneously testosterone propionate (3 mg/kg bw) and orally distilled water for 28 days to induce benign prostatic hyperplasia in male Wistar rats (GM-BPH 3 mg/kg bw).
- Group 3: Sick rats (testosterone propionate 3 mg/kg bw) and treated with an aqueous extract of C. fruticosa at a dose of 200 mg/kg bw (GM-Tcf 200 mg/kg bw) for 28 days.
- Group 4: Sick rats (testosterone propionate 3 mg/kg bw) treated with an aqueous extract of C. fruticosa at a dose of 400 mg/kg bw (GM-Tcf 400 mg/kg bw) for 28 days.
- Group 5: Diseased rats (testosterone propionate 3 mg/kg bw) treated orally with finasteride at a dose of 10 mg/kg body weight (GM-Tfin 10 mg/kg body weight) for 28 days.

Finasteride is a reference molecule used in the treatment of benign prostatic hyperplasia.

At the beginning of the experiment, all rats in each group were numbered from 1 to 5 and weighed. They were all fed identically. Throughout the experiment, the rats in the different groups were weighed weekly. At the end of the treatment, they were fasted for 12 hours and then anesthetized with ether. Blood was collected by amputation of the tip of the tail (5 mm from the tip), previously disinfected with ethanol at 96°C, and then distributed into several tubes

containing EDTA as well as into dry tubes. The rats were then sacrificed with pentobarbital (50 mg/kg body weight) by intraperitoneal injection.

The studies were conducted in accordance with the ethical guidelines of the Committee for the Control and Surveillance of Experiments on Animals as described in the "European Community Guidelines, EEC Directive 86/609/EEC" on the use of animals in laboratories.

2.7. Determination of hematological and biochemical parameters

Blood samples in dry EDTA tubes were used for hematological analysis. The blood samples in dry tubes were centrifuged at 3000 rpm for 5 minutes to obtain serum, which was stored at -20 °C for biochemical analysis.

Hematological analyses were performed using a Sysmex KX-21 automated hematology analyzer (Canada). They included red blood cell counts, platelets, white blood cells, neutrophils, lymphocytes, hemoglobin concentrations, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).

Biochemical analyses were performed using the Cobas integra 400 automated analyzer (France), according to the corresponding reagent data sheets. These analyses included the determination of creatinine, transaminases, albumin, triglycerides, and glycerol.

2.8. Determination of prostate specific antigen levels

For the detection of Prostate Specific Antigen (PSA) level, a Rapid Detection Test (RDT) kit was used (Accu-Tell® PSA, China). This test was based on a rapid chromatography immunoassay from serum following the manufacturer's instructions.

2.9. Statistical analyses

Results were expressed as mean with standard error of the mean (SEM). Differences between animal groups were investigated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test, using Graph Pad Prism software (version 8.4.3). Values were considered significant at the $p < 0.05$ level.

3. Results

3.1. Phenolic compound contents of the aqueous extract of C. fruticosa

Table 1 summarizes the different phenolic compound contents of the aqueous extract of Cordyline fruticosa. Thus, it appears that within the aqueous extract of C. fruticosa, the total phenol content was estimated at 92.34 ± 2.87 mg EAG/g of extract for an overall total flavonoid content of 15.21 ± 2.59 mg EQ/g of extract.

Table 1 Phenolic compound contents of the aqueous extract of Cordyline fruticosa

Plant extract	Total phenols (mg EAG/g)	Total flavonoids (mg EQ/g)
C. fruticosa	92.34 ± 2.87	15.21 ± 2.59

3.2. Effects of Cordyline fruticosa aqueous extract on rat weights and prostate weights

As table 2 shows, at the end of the experiment, the final average weights of the treated animals varied between293.14 ± 2.85g (GM-Tcf 200 mg/kg bw)And295.67 ± 3.98g (GM-Tcf 400 mg/kg bw)The average weight gains for these two batches after treatment were respectively24.98 g and 30.09 g. As for the final mean weights of the normal group and the diseased group, they were 320.15 ± 3.66 g and 221.81 ± 5.03 g respectively, the mean weights of the prostates were between 260.02 ± 2.35 g (normal group) and 423.08 ± 3.94 g (disease and untreated group, GM-HBP 3 mg/kg bw). In addition, very highly significant differences ($p < 0.0001$) were noted between the final mean weight and the prostate weight of the rats in the diseased and untreated group and those of the rats in the normal group.However, treatments provided with the aqueous extract of Cordyline fruticosa (200 mg/kg bw and 400 mg/kg bw) and finasteride (10 mg/kg bw), significantly improved these different weights, compared to those of the sick and untreated group. Nevertheless,It should be noted that during the treatments the highest body weight value (306.12 ± 4.18)and the smallest prostate weight value (313.53 ± 4.50)were recorded in rats treated withaqueous extract of Cordyline fruticosa (400 mg/kg).

3.3. Effects of Cordyline fruticosa aqueous extract on hematological parameters

L'induction of BPH in rats caused a very highly significant decrease ($p < 0.0001$) in the values of hemoglobin (HB) (9.08 ± 0.45) and Mean cell volume (MCV) (50.23 ± 0.61) and a moderately significant decrease ($p \leq 0.01$) in the value of red blood cells (RBC) (6.24 ± 0.55) while those of white blood cells (WBC) (18.84 ± 0.85), lymphocytes (LYM) (92.91 ± 1.26) and neutrophils (NEU) (23.01 ± 0.48) experienced a very highly significant increase ($p < 0.0001$) while blood platelets (PLT) (835.24 ± 28.14) moderately increased ($p \leq 0.01$)) compared to the normal group (Table 3).

Table 2 Effects of aqueous extracts of Cordyline fruticosa on rat weights and prostate weights

Groups of rats	Initial weight of rats (g)	Final weight of rats (g)	Weight gain (g)	Prostate weight (mg)
GN (Normal group)	280.30 ± 6.5	320.15 ± 3.66	39.85	260.02 ± 2.35
GM-HBP (diseased group)	251.67 ± 1.50	221.81 ± 5.03****	-29.86****	423.08 ± 3.94****
GM-Tcf 200 mg/kg bw	268.16 ± 4.02	293.14 ± 2.85####	24.98####	315.03 ± 1.62####
GM-Tcf 400 mg/kg bw	265.58 ± 5.70	295.67 ± 3.98####	30.09####	312.25 ± 2.51####
GM-Tfin 10 mg/kg bw	262.16 ± 3.98	284.39 ± 3.71####	22.23####	316.86 ±4, 83####

Values are means ± Standard Errors of the Mean (m ± SEM) with n = 5 rats per group. GN: no diseased rats not treated with the plant extract; GM-HBP 3 mg/kg bw:sick and untreated rats; GM-Tcf 200 mg/kg bw:sickened rats treated with C. fruticosa plant extract at 200 mg/kg bw; GM-Tcf 400 mg/kg bw: sickened rats treated with C. fruticosa plant extract at 400 mg/kg bw; GM-Tfin 10 mg/kg bw: sickened rats treated with finasteride at 10 mg/kg bw; Batch GM-HBP VS batch GN **** $p<0.0001$. Batchs-Tcf VS batch GM-HBP ####$p < 0.0001$.

Table 3 Effects of aqueous extract of Cordyline fruticosa on hematological parameters

Hematological parameters	GN (Normal group)	GM-HBP (Diseased group)	GM-Tcf 200 mg/kg bw	GM-Tcf 400 mg/kg bw	GM-Tfin 10 mg/kg bw
HB (g/dL)	12.07 ± 0.25	9.08 ± 0.45****	11.61 ± 0.40####	11.96 ± 0.28####	11.85 ± 0.34####
GR (x106/µL)	8.8 ± 0.51	6.24 ± 0.55**	7.08 ± 0.54	8.86 ± 0.32##	9.13 ± 0.71####
GB (x103/µL)	13.78 ± 0.25	18.84 ± 0.85****	13.61 ± 0.23####	13.75 ± 0.42####	13.20 ± 0.56####
MCV (fl/cel)	55.77 ± 0.23	50.23 ± 0.61****	54.85 ± 0.70####	55.27 ± 0.67####	55.56 ± 0.63####
MCHC (g/dL)	23.61 ± 0.56	23.13 ± 1.41	23.84 ± 1.04	23.7 ± 0.71	23.41 ± 0.27
LYM (%)	83.9 ± 0.74	92.91 ± 1.26****	83.2 ± 1.36####	83.85 ± 0.58####	83.59 ± 1.23####
PLT(x103/µL)	736.1 ± 20.09	835.24 ± 28.14**	740.38 ± 29.08##	738.51 ± 26.31##	739.41 ± 21.08##
NEW (%)	18.6 ± 0.76	23.01 ± 0.48****	20.08 ± 0.92###	19.09 ± 0.18####	22.04 ± 0.29

Values are means ± Standard Errors of the Mean (m ± SEM) with n = 5 rats per group; GN: no diseased rats not treated with the plant extract; GM-HBP 3 mg/kg bw: sick and untreated rats; GM-Tcf 200 mg/kg bw: rats made sick and treated with C. fruticosa plant extract at 200 mg/kg bw; GM-Tcf 400 mg/kg bw: rats made sick and treated with C. fruticosa plant extract at 400 mg/kg bw; GM-Tfin 10 mg/kg bw: rats made sick and treated with finasteride at 10 mg/kg bw; Batch GM-HBP VS batch GN **$p<0.01$ and ****$p<0.0001$. Batchs –Tcf VS batch GM-HBP ##$p < 0.01$, ###$p < 0.001$ and ####$p < 0.0001$.

3.4. Effects of Cordyline fruticosa aqueous extract on biochemical parameters

In general, induction of BPH led to an increase in the values of the biochemical parameters studied (Table 4). This increase was very highly significant ($p \leq 0.0001$) for the activity of Aspartate Aminotransferase (ASAT) and significant ($p \leq 0.05$) for that of triglycerides (TG) and that of cholesterol (CHOL).

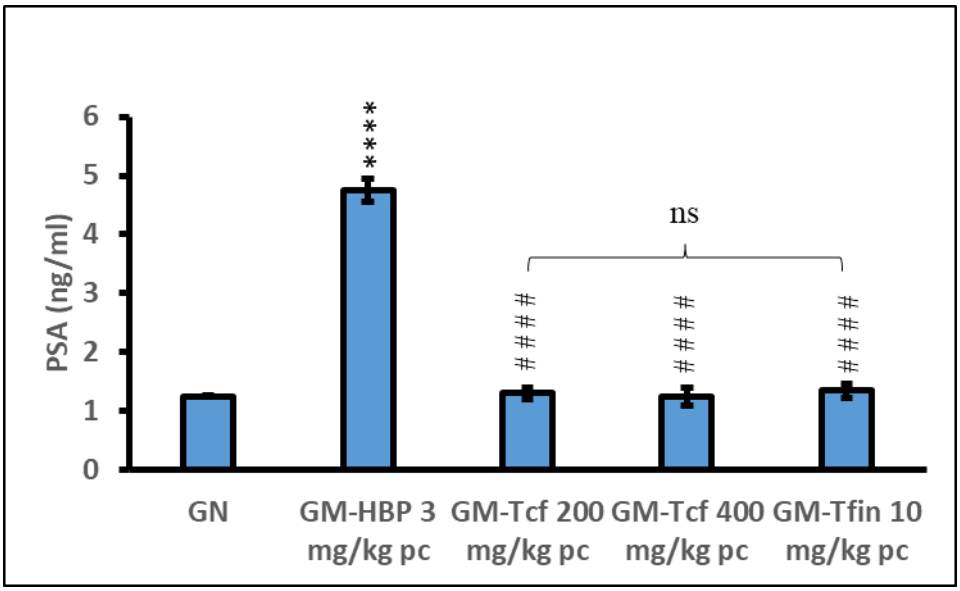
However, treatment of animals from diseased batches with different doses of C. fruticosa, and with finasteride, resulted in a decrease in the values of the parameters studied. Indeed, after treatment, the ASAT values recorded in particular for GM-Tcf 200 mg/kg bw (51.15 ± 0.51), for GM-Tcf 400 mg/kg bw (50.06 ± 0.39) and for GM-Tfin (58.71 ± 0.25) were very highly significant ($p \leq 0.0001$) compared to that of the diseased and untreated group (75.28 ± 0.36). The triglyceride values of GM-Tfin (95.92 ± 2.97)and GM-Tcf 400 mg/kg bw (73.14 ± 1.98)were, respectively, highly significant ($p \leq 0.01$) and significantly ($p \leq 0.05$) higher than that of GM-HBP (81.02 ± 2.26). Also, a significant increase ($p < 0.01$) cholesterol was recorded in rats treated with the reference molecule and those treated with the aqueous extract of C. fruticosa at 400 mg/kg bw.

Table 4 Effects of Cordyline fruticosa aqueous extract on biochemical parameters

Biochemical parameters	GN (Normal group)	GM-HBP (Diseased group)	GM-Tcf 200 mg/kg bw	GM-Tcf 400 mg/kg bw	GM-Tfin 10 mg/kg bw
AST (U/L)	49.01 ± 0.21	75.28 ± 0.36****	51.15 ± 0.51####	50.06 ± 0.39####	58.71 ± 0.25####
ALT (U/L)	90.8 ± 3.35	95.12 ± 2.59	88.63 ± 4.11	91.92 ± 2.86	93.31 ± 3.87
CREAT (mg/dL)	0.32 ± 0.05	0.35 ± 0.04	0.28 ± 0.06	0.33 ± 0.04	0.33 ± 0.05
ALB (g/dL)	4.12 ± 0.25	4.24 ± 0.46	3.28 ± 0.32	3.54 ± 1.16	4.38 ± 1.39
TG (mg/dL)	72.35 ± 3.98	81.02 ± 2.26*	74.08 ± 3.02	73.14 ± 1.98#	95.92 ± 2.97###
CHOL (mg/dL)	79.08 ± 4.28	92.25 ± 5.01*	88.62 ± 3.13	78.93 ± 4.18#	105.91 ± 2.87#

Values are means ± Standard Errors of the Mean (m ± SEM) with n = 5 rats per group; GN: no diseased rats not treated with the plant extract; GM-HBP 3 mg/kg bw:sick and untreated rats; GM-Tcf 200 mg/kg bw:rats made sick and treated with C. fruticosa plant extract at 200 mg/kg bw; GM-Tcf 400 mg/kg bw: rats made sick and treated with C. fruticosa plant extract at 400 mg/kg bw; GM-Tfin 10 mg/kg bw: rats made sick and treated with finasteride at 10 mg/kg bw; Batch GM-HBP VS batch GN *p<0.05 and ****p<0.0001. Batchs-Tcf VS batch GM-HBP #p < 0.05, ###p < 0.001 and ####p < 0.0001.

3.5. Effect of *Cordyline fruticose* aqueous extract on prostate specific antigen



GN: no diseased rats not treated with plant extract; GM-HBP 3 mg/kg bw: sick and untreated rats; GM-Tcf 200 mg/kg bw:rats made sick and treated with C. fruticosa plant extract at 200 mg/kg bw; GM-Tcf 400 mg/kg bw: rats made sick and treated with C. fruticosa plant extract at 400 mg/kg bw; GM-Tfin 10 mg/kg bw: rats made sick and treated with finasteride at 10 mg/kg bw. Batch GM-HBP VS batch GN ****p<0.0001. Batchs-Tcf VS batch GM-HBP ####p < 0.0001.

Figure 2 PSA levels in different batches of rats after administration of the aqueous extract of *Cordyline fruticose*

Induction of benign prostatic hyperplasia in batch 2 rats (GM-HBP)caused a strong increase in PSA content (4.75 ± 0.19 ng/ml) in these rats, with a very highly significant difference($p < 0.0001$)compared to the normal group (1.25 ± 0.01

ng/ml). After administration of the aqueous extract of Cordyline fruticosa at doses of200 mg/kg bw and400mg/kg bw in sick rats, the mean PSA levels of these animals were respectively 1.3 ± 0.1 ng/ml and 1.24 ± 0.15 ng/ml at the end of treatment. These values indicate a very highly significant decrease($p < 0.0001$)compared to that of the diseased and untreated batch.

Under the effect of the reference molecule (finasteride) at the dose of10 mg/kg bw, a PSA content of 1.35 ± 0.12ng/ml, reflecting a very highly significant decrease ($p < 0.0001$) compared to the GM-HBP 3 mg/kg bw group, was observed at the end of the experiment in rats. However, no significant difference was reported between the PSA levels of the finasteride-treated group and the groups treated with the aqueous extract of C. fruticosa (Figure 2).

4. Discussion

A very highly significant increase in prostate weights was observed in the diseased (GM-BPH) and untreated group, compared to the normal control group (GN). This elevation in prostate weight indicates that benign prostatic hyperplasia (BPH) was indeed induced in this study [15]. According to Wang & Olumi (2011) [16], the increase in prostate weight would be due to an increase in the number of abnormal cells in the prostatic tissues. However, administration of the aqueous extract of Cordyline fruticosa to rats with BPH significantly reduced their prostate weights. This decrease in prostate weights suggests that this extract inhibits the hyperplastic progression of the prostate in BPH rats induced by testosterone propionate. Our results are similar to those of Gupta et al. (2004) [17] who reported that C. bonduc seeds possessed significant anticancer property in a study conducted on albino mice. Similarly,Shan et al. (2022) [9], reported that the C. bonduc seed extracthadinhibitory and antiproliferative effects on the development of BPH. Regarding hematological and biochemical parameters, the administration of testosterone propionate resulted in significant changes in these parameters, compared to those of rats in non-diseased and untreated groups (control). Identical results had already been reported by Anselmo et al. (1983) [18] following a study evaluating the effect of testosterone propionate on hematological and biochemical parameters. However, the aqueous extract of C. fruticosa allowed the values and concentrations of these hematological and biochemical parameters to be brought back to normal. This observation could be explained by the richness of this medicinal plant in different groups of secondary metabolites. Indeed, our first work on a decoction of C. fruticosa had made it possible to highlight the presence of phenolic compounds, alkaloids, saponosides then sterols and poly terpenes [19]. Some of these chemical compounds, notably flavonoids and sterols and terpenes, are known for their anti-BPH effects [20].

The significantly elevated serum PSA levels in the untreated diseased control group, compared to the healthy rat group (GN), suggest the development of BPH in this group of rats and demonstrates successful induction. These levels were significantly reduced with the administration of C. fruticosa extract in the groups of rats receiving doses of 200 mg/kg bw and 400 mg/kg bw. This therefore suggests an excellent potential of this plant in the management of BPH.

5. Conclusion

Our work allows us to conclude that the aqueous extract of Cordyline fruticosa contains phenolic compounds. Also, this extract has an inhibitory effect on benign prostatic hyperplasia induced by testosterone in rats. The aqueous extract of Cordyline fruticosa also improved the hematological and biochemical parameters evaluated during benign prostatic hyperplasia. All these very promising results justify the frequent use of this medicinal plant for the treatment of certain diseases andurinary tract infections. However, further work should be carried out to assess the toxicity of the extract, isolate and then characterize the molecules responsible for these biological activities, in order to formulate a traditional medicine product accessible to all.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Also, all procedures performed in studies involving animals were in accordance with the ethical standards.

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