

Protective Effect of the Ethanolic Extracts of Leaves of *Chuquiraga spinosa* Less and *Baccharis genistelloides* on Benign Prostatic Hyperplasia in Rats

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ABSTRACT

Introduction: Benign prostatic hyperplasia (BPH) is one of the most common conditions affecting middle-aged men, with oxidative stress postulated as an important factor in its development. **Objective:** To evaluate the protective effect of the association of the ethanolic extract of *Chuquiraga spinosa* (ChS) and *Baccharis genistelloides* (BaG) on benign prostatic hyperplasia in rats. **Materials and Methods:** Sixty-six male rats were randomized into groups: 1) Inductor Group: Testosterone enanthate (ET) 25 mg/Kg on days 8 and 14 intramuscularly. 2) Group P80: Polysorbate-80 3% 10 mL / Kg. 3) Positive Group: ET + Dutasteride (DU) 0.5 mg / Kg, 4) ET + ChS 250, 5) ET + ChS 500, 6) ET + BaG250, 7) ET + BaG 500, 8) ET + ChS 250 + BaG 250, 9) ET + ChS 500 + BaG 500, 10) ET + ChS 250 + BaG 500, 11) ET + ChS 500 + BaG250. PSA, nitric oxide (NO) and malondialdehyde (MDA), prostate dimensions by ultrasound and histopathological findings were quantified as main markers. **Results:** Lower levels of NO, MDA and PSA were observed in all groups treated with extract compared to the Inductor group ($p < 0.01$), with the reductive capacity of the mixture ChS 250 + BaG 500 and ChS 500 + BaG 250 compared with Dutasteride ($p < 0.05$). The volume of the prostate was lower in the Dutasteride, P80 and ChS250 + BaG500 groups compared to the inducer ($p < 0.05$). Regarding the histopathological study, it was observed that the association of variable doses (ChS250 + BaG500 and ChS500 + BaG250) had a better protective effect. **Conclusion:** Under experimental conditions, the association of the extracts of *Chuquiraga spinosa* and *Baccharis genistelloides* has an effect in the protection of BPH induced with testosterone, which could be linked to its antioxidant capacity of the extracts.

Key words: Benign prostatic hyperplasia, Testosterone, *Chuquiraga spinosa*, *Baccharis genistelloides*, Dutasteride.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is one of the most common conditions affecting middle-aged men. Since 2003, an increase in global prevalence has been reported, currently affecting around 1.1 billion individuals. Up to 25% of men aged 50 to 65 years have lower urinary tract symptoms such as nocturia, urgency, frequency, a feeling of not completely emptying the bladder, effort to urinate and weak urinary flow these discomforts can significantly affect the quality of life. The costs of BPH treatments are based on direct costs (medications, procedures, images, medical consultations), indirect costs (loss of income) and intangibles (pain and suffering).^{1,2}

The most effective pharmacological treatments for BPH with symptoms of the lower urinary tract include alpha-1 adrenergic blockers and 5-alpha reductase inhibitors. Perhaps, up to 30% of patients do not achieve a sufficient improvement of symptoms with medication and have adverse effects such as rhinitis, orthostatic hypotension,

headache, iris prolapse, retrograde ejaculation, livid reduction, erectile dysfunction and gynecomastia; In addition to the high cost of treatments, the idea has been reinforced towards the search and use of "safer" plant extracts.^{3,4}

Chuquiraga and *Baccharis* are a large genus of the Asteraceae family with more than 500 species widely distributed in America, but with greater diversity in the Andes. *Chuquiraga spinosa* Less (ChS) is an herbaceous plant widespread in the western Andes of Peru, between 3000 and 4500 m altitude, it is used in folk medicine for the treatment of inflammatory diseases (mainly against prostatic diseases).⁵

The phytochemical studies of *Chuquiraga spinosa* Less have revealed that it contains 9 types of flavonoids (quercetin-3-O-glucuronide, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-glucuronide, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, isorhamnetin-3-O-glucuronide, isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside) and a phenolic compound (p-hydroxyacetophenone), flavonoids

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such as isorhamnetin-3-O-glucurodino has anti-inflammatory activity *in vitro*.^{6,7} Studies have demonstrated a protective effect against prostate cancer induced with N-methyl-nitrosourea (NMU) in rats and cytotoxicity in prostatic carcinoma cell lines (DU-145),⁸ which could be explained by the capacity anti-inflammatory and antioxidant polyphenols.

Baccharis contains around 77 species in Peru, 17 of which are endemic.⁹ *Baccharis genistelloides* is used traditionally in the Andean region of Peru, as an infusion and / or maceration of leaves and stems, for diabetes, headaches, muscle aches, as a diuretic, to avoid postpartum complications, as an anti-inflammatory of the urinary tract, liver, gallbladder and for baldness.¹⁰ The ethanolic extract of *Baccharis genistelloides* has a chemopreventive effect on colon cancer and gastric cancer;¹¹ the crude extract has an antioxidant effect, the aqueous extract showed antiviral activity against herpes simplex type I (HSV-1) and the vesicular stomatitis virus (VSV), antiarthritic effect in addition to hypoglycemic and hypotriglyceridemia action.¹² Investigations of the chemical components of *Baccharis genistelloides* allowed to isolate, flavones called eupatrin, cirsimaritin, cirsiolol, hispidulin and genkwanin, together with apigenin, believed to be the main substances of this plant.¹³

Considering the previous investigations, it was proposed to associate the ethanolic extract of *Chuquiraga spinosa* Less and *Baccharis genistelloides* searching for a protective effect in the benign prostatic hyperplasia, as well as to identify some findings of toxicity.

MATERIAL AND METHODS

Chemicals

Testosterone enanthate (ET), thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium nitrite, sulfanilic acid, phosphoric acid, N-1-Naphthylethylenediamine were purchased from Sigma-Aldrich, USA. Others, chemicals were of analytical grade.

Preparation of the ethanolic extract of *Chuquiraga spinosa* and *Baccharis genistelloides*

Chuquiraga spinosa Less (ChS) known as "huamanpinta" was collected in Huamanga, Ayacucho, Peru and *Baccharis genistelloides* known as "carqueja" (BaG) was collected in Huancayo, Junín, Peru. The taxonomic identification was made in the National Herbarium of the Universidad Nacional Mayor de San Marcos, Lima, Peru. Each plant was dried at 40°C and pulverized in a blade mill. Material plants were soaked into 96% ethanol for 7 days. Next, the extract was filtered and concentrated obtaining a solid residue, its final weight was recorded, and it was kept refrigerated until further use.

Qualitative phytochemical screening

The presence of phytochemical constituents such as alkaloids, terpenoids, quinone, flavonoids, tannins, saponins, steroids and phenolic compounds were determined with standardized phytochemical methods.¹⁴

Animals

Male Holtzman albino rats were used with an average weight of 200 ± 20 g, from the National Institute of Health (INS) - Peru, set for 5 days in large ventilated cages with food and water *ad libitum*. The study was conducted at the Bioterio and Pharmacology laboratory of the Faculty of Medicine of the UNMSM. The rats were kept in periods of light of 12 hours / dark cycle and a temperature of 21 ± 2°C.

Acute toxicity of the ethanolic extracts of *Baccharis genistelloides* and *Chuquiraga spinosa* in rats

The ethanolic extracts were evaluated according to the guide number 423 of the Organization for economic cooperation and development (OECD), where the limit dose test of 2000 mg / Kg was used as a follow-up protocol (Organization of Economic Cooperation and Development, 2002).¹⁵

Twenty Holtzman male rats were fasted during the night before the test, with water access *ad libitum*. The rats were randomized into 4 groups of 5 animals per group. One group was used as a negative control, which was administered 10 mL / Kg of P80 orally. The other groups received orally BaG 2000, ChS 2000, BaG 2000 + ChS 2000. At the beginning of the experiment the weight of each animal was determined, the rats were observed for 4 hours to see any toxic signs, then they were observed for a period of time. 14 days to evaluate changes in behavior and other parameters such as change in body weight, food intake, motor activity, tremor, diarrhea, death, changes in eye and skin colors.

Induction of benign prostatic hyperplasia (BPH)

The induction of hyperplasia was carried out using Testosterone Enanthate (ET) with slight modifications.¹⁶ Sixty-six animals were randomly distributed in 11 groups; according to the following experimental design:

Control group: ET 25 mg / Kg on days 8 and 14 intramuscularly

Sham group: 80% 3% polysorbate 10 mL / Kg

Treated group: ET + Dutasteride (DU) 0.5 mg / Kg

ET + ChS 250 mg / Kg

ET + ChS 500 mg / Kg

ET + BaG 250 mg / Kg

ET + BaG 500 mg / Kg

ET + ChS 250 mg / Kg + BaG 250 mg / Kg

ET + ChS 500 mg / Kg + BaG 500 mg / Kg

ET + ChS 250 mg / Kg + BaG 500 mg / Kg

ET + ChS 500 mg / Kg + BaG 250 mg / Kg

At the end of the experiment the rats were weighed. Blood samples were collected to analyze the biochemical parameters. The animals were sacrificed using pentobarbital (100 mg / Kg)

Biochemical parameters

PSA was quantified by chemiluminescence in an automated immunology analyzer (MAGLUMI 1000) in serum.¹⁷ The antioxidant capacity was determined by the quantification of nitric oxide (NO), which was developed using the Griess reagent method.¹⁸ The lipoperoxidation was determined by the quantification of the malondialdehyde (MDA) production (Buege & Aust, 1978) and was analyzed in the Genesys 10 UV-VIS spectrophotometer.¹⁹

Prostate ultrasound

After the induction of HBP, a high-level system (CHISON D600 VET, JIANG SU, CHINA) was used with a linear 10 MHz transducer. Before the examinations, the rats were shaved in the lower part of the abdomen and then placed in the supine position on a heating pad. A B-mode test was performed to determine the volume of the prostate.

Histological analysis

For the histological analysis, the prostates were removed and fixed in 10% formalin. After being processed, both lobes were embedded in paraffin. Sections were cut at 5 µm and stained with H & E (hematoxylin and eosin). The plates were examined under a light microscope (Olympus BX51).

Statistical analysis

Study parameters such as weight, prostate specific antigen (PSA), Nitric Oxide (ON), were expressed as mean ± standard deviation (SD). The variance homogeneity was determined by the Levene test and normality by the Wilk-Shapiro W statistics. The data that followed a normal distribution were subjected to a one-way analysis of variance (ANOVA) followed by the multiple comparison method. of Tukey, otherwise the Kruskal-Wallis test was applied. The F test was performed to analyze intra- and intergroup variability. A value of $p < 0.05$ was considered statistically significant in all cases. The data was analyzed using SPSS v. twenty-one.

Ethical considerations

The Guide of ethics for experimental animals was considered Guide for the Care and Use of Laboratory Animals. For this the present work is based on the 3R's: refinement, reduction and replacement. (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). During the experimental process, international ethical principles for research with laboratory

animals were respected. The rats were euthanized by intraperitoneal administration of pentobarbital (100 mg / kg), in order to achieve a rapid and peaceful death.²⁰

RESULTS

Table 1 shows the secondary metabolites present in the ethanolic extracts of the aerial parts of *Chuquiraga spinosa* and the whole plant of *Baccharis genistelloides*, such as the flavonoids followed by terpenes and alkaloids.

Table 2 shows lower levels of NO, MDA and PSA in all the groups treated with extract compared to the Inductor group ($p < 0.01$), with the reductive capacity of the mixture ChS 250 + BaG 500 and ChS 500 + BaG standing out 250 compared with Dutasteride ($p < 0.05$).

In Figure 1, the evolution of body weights is appreciated, the start and end are compared, as well as the variation percentage; demonstrating that the weight variation percentage was lower in the groups with mixture of both extracts in comparison with the inductor and dutasteride groups ($p < 0.05$). Figure 2 shows the effect on prostate volume, being lower in the Dutasteride, P80 and ChS250 + BaG500 groups compared to the inducer ($p < 0.05$).

Values expressed as mean + standard deviation (n = 6) # ANOVA test ($p < 0.01$); P80: 3% Polysorbate 80; ChS250 and ChS500: 250 mg / Kg, 500 mg / Kg of extract of *Chuquiraga spinosa* and BaG 250, BaG 500: 250 mg / Kg, 500 mg / Kg of extract of *Baccharis genistelloides* respectively. * Tukey Test ($p < 0.05$) Vs inductor group.

Table 1: Phytochemical constituents of the ethanolic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides*.

Phytochemicals	Tests	EE -ChS	EE - BaG
Alkaloids	Mayer	+	+
	Dragendorff	+	+
	Wagner	+	+
Flavonoids	Shinoda	+	+
Quinone	Bornträger	-	-
Phenols	Ferric chloride	+	+
Saponins	Froting	+	+
Tannins	Gelatin	+	+
Terpenes and steroids	Lieberman - Burchardat	+	+

ChS: *Chuquiraga spinosa*; BaG: *Baccharis genistelloides*; EE: Ethanolic extract. (+) Present (-) Absent

Table 2: Main markers biochemical markers in rats treated with the ethanolic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides*.

Tratamiento	NO (µg/L)	MDA (µmol/mL)	PSA (ng/mL)
Dutasteride	50.48 ± 0.86	07.20 ± 0.35	0.39 ± 0.05
Inductor	92.80 ± 7.43	12.72 ± 0.82	1.07 ± 0.04
P80	48.55 ± 5.53 ^(a)	07.35 ± 0.17 ^(a)	0.42 ± 0.08 ^(a)
ChS 250	78.30 ± 6.48 ^(a)	09.53 ± 0.07 ^(a)	0.84 ± 0.04 ^(a)
ChS 500	71.40 ± 5.59 ^(a)	09.31 ± 0.78 ^(a)	0.80 ± 0.09 ^(a)
BaG 250	69.20 ± 3.49 ^(a)	10.63 ± 0.65 ^(a)	0.88 ± 0.13 ^(a)
BaG 500	54.80 ± 3.27 ^(a)	09.98 ± 0.32 ^(a)	0.69 ± 0.07 ^(a)
ChS 250 + BaG 250	49.60 ± 8.29 ^(a)	08.82 ± 0.18 ^(a)	0.56 ± 0.04 ^(a)
ChS 500 + BaG 500	43.00 ± 8.37 ^(a)	08.72 ± 0.17 ^(a)	0.44 ± 0.25 ^(a)
ChS 250 + BaG 500	41.00 ± 8.94 ^(a,b)	08.75 ± 0.84 ^(a)	0.46 ± 0.25 ^(a)
ChS 500 + BaG 250	35.00 ± 5.00 ^(a,b)	08.15 ± 0.62 ^(a)	0.40 ± 0.21 ^(a)
Coefficiente F/p#	41.2/ $p < 0.01$	42.8/ $p < 0.01$	14.9/ $p < 0.01$

Values expressed as mean + standard deviation (n = 6) # ANOVA test ($p < 0.01$).

NO: Nitric oxide; MDA: malondialdehyde; PSA: Prostate specific antigen; P80: 3% Polysorbate 80; ChS250 and ChS500, is 250 mg / Kg, 500 mg / Kg of *Chuquiraga spinosa* extract and BaG 250, BaG 500, is 250 mg / Kg, 500 mg / Kg of *Baccharis genistelloides* extract respectively.

(a) Tukey Test ($p < 0.05$) Vs inductor group

(b) Tukey Test ($p < 0.05$) Vs dutasteride group

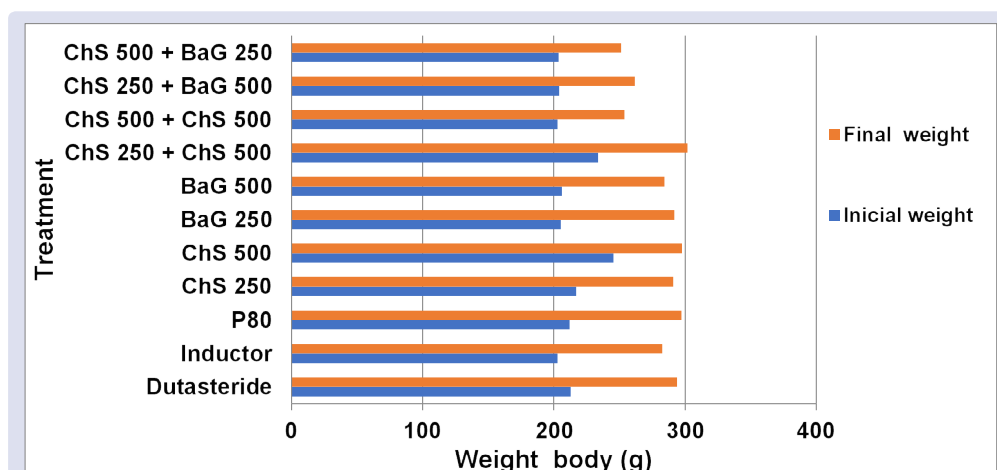


Figure 1: Evolution of weights of rats treated with ethanolic extract of *Chuquiraga spinosa* and *Baccharis genistelloides*. P80: 3% Polysorbate 80; ChS250 and ChS500: 250 mg/Kg, 500 mg/Kg of extract of *Chuquiraga spinosa* and BaG 250, BaG 500: 250 mg/Kg, 500 mg/Kg of extract of *Baccharis genistelloides* respectively. * Tukey Test ($p < 0.05$) Vs inductor group, Vs dutasteride group.

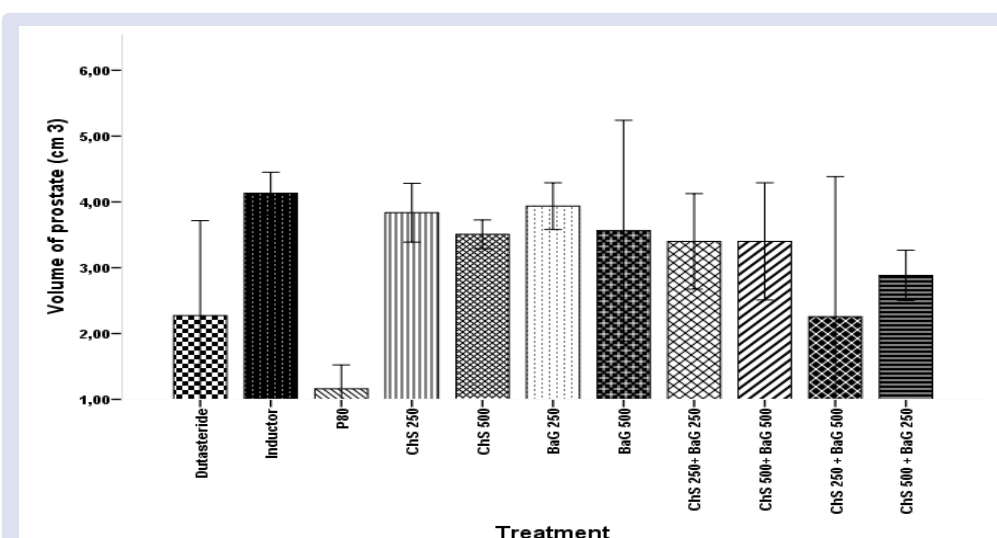


Figure 2: Effect on prostate volume in rats treated with ethanolic extracts of *Chuquiraga spinosa* y *Baccharis genistelloides*.

The acute toxicity effect of the ethanolic extracts of *Chuquiraga spinosa* and *Baccharis genistelloides* was determined according to the guide 423 of the OECD, where the limit dose of 2000 mg / Kg was used. No signs or symptoms of toxicity were observed nor were there deaths after oral administration of the extracts. The general behavior was observed first for a short period of 4 hours followed by a period of 14 days, there were no changes in behavior and other parameters such as body weight, food intake, motor activity, tremor, diarrhea, changes in color the eyes and skin.

The sham group (P80) showed a regular lumen lined by a single layer of cubic epithelium, and a scant fibromuscular matrix (Figure 3A). In contrast, the control group (inductor) presented an irregular lumen with multiple papillary projections covered by cylindrical epithelium with evident loss of polarity. The stroma showed mild fibrosis and numerous congestive blood vessels (Figure 3B). In the treated group (dutasteride) (Figure 3C), the treatment prevented the development of morphological changes in both the glandular epithelium and the stroma. Compared with the control group (inducer), the isolated administration of *Chuquiraga spinosa* partially attenuated the development of histological changes in the prostate gland, especially the changes on the glandular

epithelium in the ChS500 group (Figures 3D and 3E). In contrast, the isolated administration of *Baccharis genistelloides* does not attenuate the development of histological changes associated with prostatic hyperplasia. The fixed-dose association of both treatments partially attenuates changes in the glandular epithelium and stroma induced by testosterone, especially in the ChS250 + BaG250 group (Figures 3F and 3G). On the other hand, the association with variable doses partially attenuates the changes both at the level of the glandular epithelium, as well as in the stroma, especially in the group ChS250 + BaG500 (Figures 3H and 3I).

DISCUSSION

Qualitative phytochemical screening (Table 1) showed that the ethanolic extracts of *Baccharis genistelloides* and *Chuquiraga spinosa* have secondary metabolites such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, terpenes and steroids, of which flavonoids and phenolic compounds have been shown antioxidant and anti-inflammatory capacity,²¹ on the other hand in the species *Baccharis genistelloides* has been reported flavonoids as 5,3', 4'-OH-6,7-OMe flavone (cirsiol), and so far It is known that the main pharmacological

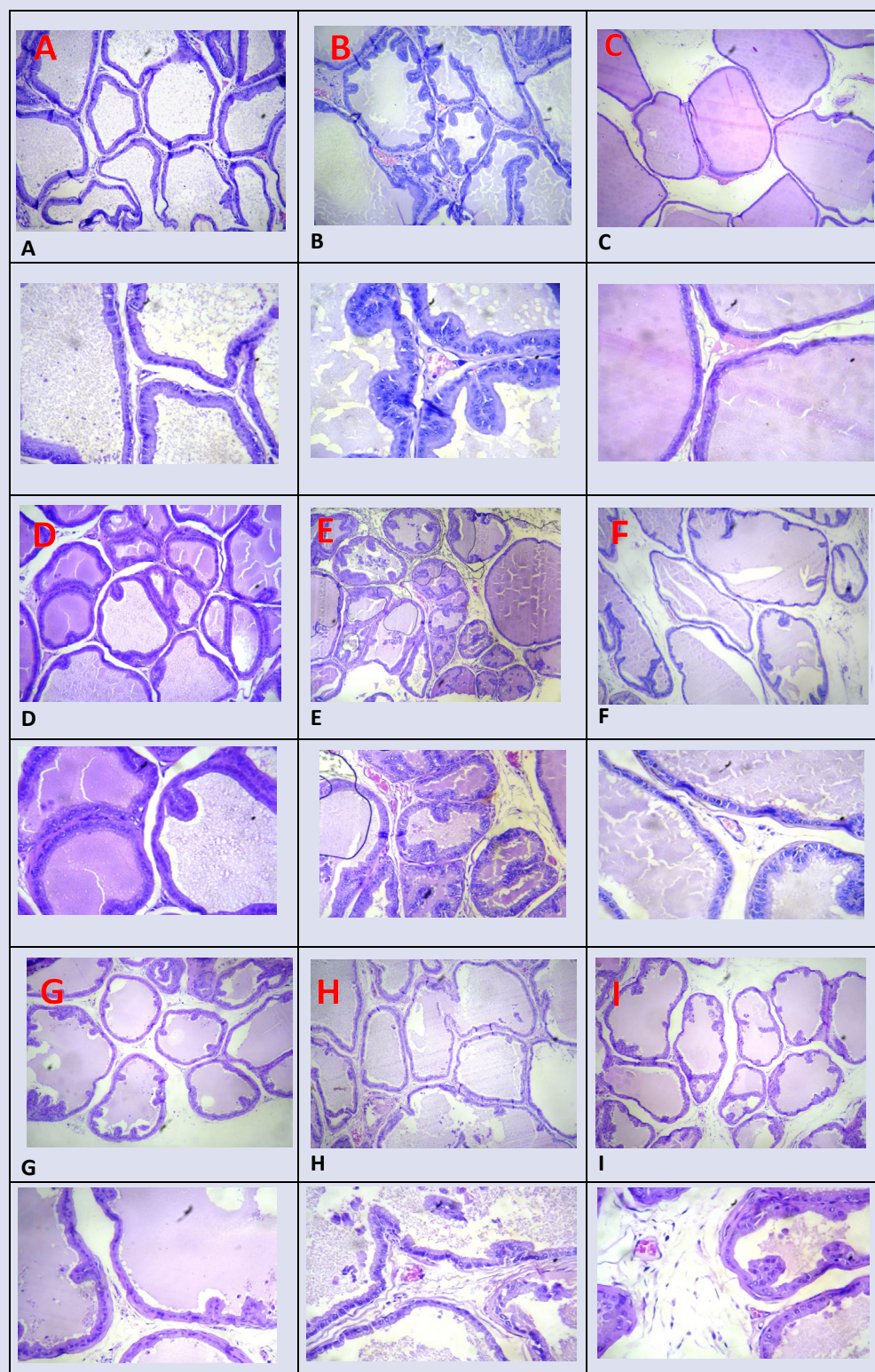


Figure 3: Histopathological evaluation of prostatic tissue. Hematoxylin and Eosin (H & E) 100X and 200X. A (P80): Structure of the prostate gland of a normal rat. B (Inductor): Histological changes associated with the administration of testosterone. C (Dutasteride): Absence of histological changes in rats treated with testosterone - dutasteride. D (ChS250): The single administration of *Chuquiraga spinosa* achieves a minimal reduction of the histological changes induced by testosterone. E (BaG250): The single administration of *Baccharis genistelloides* failed to achieve the reduction of histological changes induced by testosterone. F (ChS250 + BaG250): The combined administration at fixed low doses attenuates to a greater degree the changes induced by testosterone in comparison to the individual doses. G (ChS500 + BaG500): The combined administration at fixed high doses partially attenuates the changes induced by testosterone. H (ChS250 + BaG500): The combined administration in variable doses attenuates to a greater degree the changes induced by testosterone in comparison to the fixed doses. I (ChS500 + BaG250): The combined administration at variable doses attenuates to a greater degree the changes induced by testosterone in comparison to the fixed doses.

actions of the *Baccharis* genus are mainly due to the presence of flavonoids and terpenes.²²

The imbalance between free radicals and antioxidants was postulated as an important factor in the development of BPH.^{23,24} In patients with BPH, it has been reported high levels of Malondialdehyde (MDA), it is also shown that nitric oxide (NO) is involved in the activation of mutation of K-ras genes, angiogenesis and lipoperoxidation in colonic tumors.^{25,26} In our investigation, a significant decrease ($p < 0.05$) of the ON and MDA levels was observed (Table 2) in all the groups treated with extract compared to the Inductor group, being better the mixtures ChS 250 + BaG 500 and ChS 500 + BaG 250, evidencing an antioxidant effect; that could be explained, at least in part, by the presence of flavonoids and phenolic compounds present in the extracts (Table 1) and that were also identified by other researchers. In addition, it has been shown that flavonoids, due to their antioxidant capacity, have a beneficial effect on prostate cancer cells.²⁷

The levels of serum prostate antigen (PSA) are correlated with the volume of prostate in BPH.²⁸ In this investigation PSA levels decreased significantly ($p < 0.05$) compared to the inducer group, which would indicate a beneficial effect of the extracts on the protection of HPB, which is better with the variable dose of ChS 500 and BaG 250, where the values reached levels similar to that of the reference drug dutasteride.

Testosterone has been widely used as an inducer of benign prostatic hyperplasia (BPH) in animal models in order to test the protections of various natural products.²⁹ Hyperplasia models have been characterized by increased prostate volume, as well as alterations in prostatic tissues including enlargement of the glandular cavity, proliferation of prostatic epithelium and stromal cells and infiltration of inflammatory cells, being a pathological index of BPH.³⁰ In this study, all animals that received testosterone developed BPH, except for the group that received dutasteride (Figure 3C). The isolated administration of ChS minimally reduces the histological changes induced by testosterone, predominantly those that occur on the glandular epithelium (Figure 3D). In contrast, the isolated administration of BaG fails to attenuate the histological changes induced in this animal model (Figure 3E). The association of ChS and BaG is able to attenuate to a greater degree the histological changes induced by testosterone, when compared with those groups that receive the isolated administration of both treatments, our findings show that this reduction is lower than that achieved by the dutasteride. These histopathological changes are related to the biochemical results such as PSA, MDA and ON decrease.

Although many people believe that the use of medicinal plants in the treatment of various ailments are safe, there is scientific evidence that they report the toxicity of therapeutic agents in mammals.³¹ Body weight is an indicator of great importance, since it is involved in a series of organic changes in different stages of life. Due to a variation in its value could suggest some adverse effect of drugs or chemicals if it presents a decrease of more than 10% of the initial body weight; In our research we observed in all groups, a gain in body weight greater than 19.0% until the end of the study, being slightly lower in the groups that received extracts, this could be explained by the polyphenols present that modulate the physiological and molecular pathways involved in energy metabolism, adiposity and obesity, which would not show adverse effects. On the other hand, in the oral acute toxicity study of each extract, no significant changes in behavior or death were observed. Therefore, it is considered that the lethal dose 50 (LD50) is greater than 2000 mg/Kg for the 2 extracts, thus being considered safe and of low toxicity.

CONCLUSION

Under experimental conditions, the association of the extracts of *Chuquiraga spinosa* and *Baccharis genistelloides* has an effect on the

protection of benign prostatic hyperplasia induced with testosterone, which could be due in part to the antioxidant capacity of the extracts.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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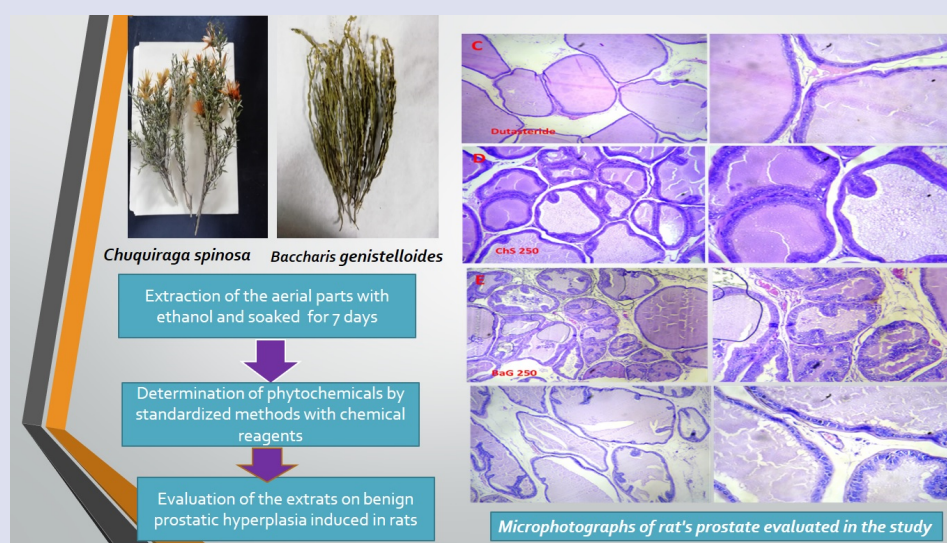
Nil.

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GRAPHICAL ABSTRACT



SUMMARY

The phytochemical studies of *Chuquiraga spinosa* Less and *Baccharis genistelloides* have revealed the presence of phenols, quinones, alkaloids and flavonoids.

Chuquiraga spinosa and *Baccharis genistelloides* has an effect on the protection of benign prostatic hyperplasia induced with testosterone.

It is considered that the lethal dose 50 (LD50) is greater than 2000 mg/Kg for the two extracts, thus being considered safe and of low toxicity.

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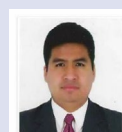
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