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Protective Effect of Ethanolic Extract of *Chuquiraga spinosa* Less (Huamanpinta) and *Senecio rhizomatus* Rusby (Llancahuasi) on Prostatic Neoplasia Induced with Testosterone, N-Methylnitrosourea (NMU) and Cyproterone in Rats

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Abstract: Background and Aim: Cancer is a public health problem in Peru and the world. The need to continue experimenting with potential protective substances against prostate neoplasia is still valid. In the present study, the protective effect of the ethanolic extract of *Chuquiraga spinosa* Less (Chs, Huamanpinta) and *Senecio rhizomatus* Rusby (Sr, Llancahuasi) in prostatic neoplasia induced by testosterone, N-methyl nitrosourea (NMU) and cyproterone in rats was determined. Materials and Methods: The phytochemical study was performed by gas chromatographic analysis, using a gas chromatograph coupled to a mass spectrometer. The protection of prostatic neoplasia in rats was done with the following distribution of experimental groups 1) normal or negative control with physiological serum 2 mL/kg; 2) positive control Testosterone 100 mg/kg + Cyproterone 50 mg/kg + NMU 50 mg/kg (TCN); 3) TCN and Chs 250 mg/kg; 4) TCN and Sr 100 mg/kg); 5) TCN and Chs 50 mg/kg and Sr 100 mg/kg; 6) TCN and Chs 250 mg/kg and Sr 100 mg/kg; 7) TCN and Chs 500 mg/kg and Sr 100 mg/kg. Results: The results indicate that the positive control presented an increase in serum catalase,

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GSH, MDA, as well as prostate size; on the other hand, prostate width x height was lower in the groups TCN and Chs 250, TCN and Chs 250 and Sr 100; TCN and Chs 500 and Sr 200 compared to TCN ($p < 0.05$). Conclusion: The association of Chs and Sr exerts a synergistic dose-independent protective effect on prostatic neoplasia by Testosterone, NMU and Cyproterone in rats.

Key words: *Chuquiraga spinosa* Less · Prostate Neoplasia · Chemopreventive *Senecio rhizomatus* Rusby

INTRODUCTION

Due to its high mortality and disabling effects, cancer is a public health issue in both Peru and the rest of the world. Lung and bronchial cancer and prostate cancer are the two most common types of new cancer diagnoses and sex-related deaths in the United States in 2020, respectively [1]. Slow growth and tolerance to androgen deprivation are characteristics of prostate cancer [2], along with the possibility of negative and toxic repercussions. However, nearly 65% of people worldwide choose to utilize therapeutic plants and roughly 60% of cancer-fighting substances come from plants and other natural resources [3]. According to reports, 3000 plants have anticancer capabilities, with 70% coming from tropical regions [2]. In northern Peru, the plant species Chuquiraga spinosa has historically been used to treat prostate conditions [4].

Studies done in Peru looked at the potential prevention of prostate cancer in rats caused by N-methyl nitrosourea (NMU) and the ethanolic extract of Chuquiraga spinosa. Hematological, biochemical and histopathological measurements were made in the study by Arroyo *et al*. [5] and anti-inflammatory, antioxidant and antigenotoxic effects were assessed. There were significant differences in the biochemical and haematological parameters, including prostate-specific antigen, which led the researchers to the conclusion that *C. spinosa* has a protective effect on prostate cancer induced by testosterone and NMU in rats.

In addition to its strong antioxidant activity, a different study has shown that *Senecio rhizomatus* Rusby (Llancahuasi) ethanolic extract has preventive effects against breast cancer that is caused by 7,12-dimethylbenz [a] anthracene (DMBA) in female rats [6].

Chuquiraga spinosa Less (Huamanpinta) stems, leaves and flowers are used as cicatrizants, sudorifics, anti-inflammatory agents, diuretics, treatments for renal and hepatic issues and as antiseptics for the prostate and urinary system. The aerial parts of the plant have been shown to have antioxidant and anti-inflammatory effects *in vivo* and *in vitro*, as well as antifungal effects. This is because the aerial parts of the plant have a high concentration of phenolic compounds, such as flavonoids and phenolic acid derivatives [7-11].

Studies on the phytochemistry of *Chuquiraga spinosa* Less have shown that it contains nine different types of flavonoids, including quercetin-3-O-glucuronide, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaemperol-3-O-glucuronide, kaempherol-3-O-glucoside, isorhamnetin-3-O-glucuronide, isorham Lastly, studies have shown that the methanolic extract has antioxidant, anti-inflammatory and antifungal action [12].

Senecio rhizomatus Rusby, also known as Llancahuasi, is a perennial herb with an upright, hairy rhizome that is 25-35 cm long. It can be found in the Puna Silvestre region's damp stony or rocky soils. Alkaloids, flavonoids and the absence of quinones are claimed to be present. It is mostly used to treat wounds, menstrual cramps, kidney infections, hepatic colic and the flu [13]. Due to the presence of a hydroxylated metabolite benzene with a variety of biological actions in this species, including antioxidant, neuroprotective, immunomodulatory and anti-inflammatory effects, 1,4-benzenediol linked to the hydroquinone molecule has been identified [14]. Additionally, it includes cyclopropanecarboxylic acid, a substance with antioxidant, antifungal, anticancer and antibacterial properties [15]. Totarol, a compound with antibacterial, antioxidant and anticancer properties, is present in the ethanolic extract of Llancahuasi [16].

Given the prevalence of prostate cancer and the active metabolites found in the plant species being studied, such as *Senecio rhizomatus* Rusby (*Llancahuasi*) and *Chuquiraga spinosa* Less (*Huamanpinta*), it is critical to continue the search for active compounds that have a significant inhibitory effect on prostate neoplasia in order to find potential treatments for this disease that affects the male reproductive system.

The goal of the current study is to ascertain whether *Senecio rhizomatus* Rusby (*Llancahuasi*) and *Chuquiraga spinosa* (Huamanpinta) together to prevent prostate neoplasia in a synergistic or supportive manner.

MATERIALS AND METHODS

Ethical Approval: This research was approved by the Ethics Committee of the Faculty of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos through registration No. 003-CE-UDI-FFB-2020;

during the execution of the research, the specifications proposed by the Institutional Committee for the Care and Use of Animals (CICUA, ILAR) and the current regulations of Law N 27265 on animal protection, Lima-Peru, were followed.

Study Period and Location: The study was conducted at the facilities of the Biotherium and Pharmacology Laboratory of the Faculty of Human Medicine of the Universidad Nacional Mayor de San Marcos (UNMSM), Lima, Peru, from april 2019 to March 2020.

Preparation of Ethanolic Extracts: The aerial parts of *Chuquiraga spinosa* Less and *Senecio rhizomatus* Rusby were obtained from the district of El Tambo, located at an altitude of 3260 meters above sea level, in the Province of Huancayo, located in the Department of Junín, Peru. These were dried at 40°C, then subjected to the grinding process to obtain small particles, to facilitate the release of their secondary metabolites during the maceration process with 96% ethanol for seven days; then they were filtered using a vacuum filtration pump. The liquid product of the filtration was subjected to evaporation of ethanol at 40°C, until obtaining dry residues with stable weight, which were placed in amber flask with lid and kept refrigerated at 4°C.

Analysis by gas chromatography coupled to coupled mass spectrometry (GC-MS): GC-MS analysis of the composition of the volatile fraction of the ethanolic extract of Ch L associated with Sr R was performed using a chromatograph, coupled to a mass spectrometer detector [17]. The volatile constituents of the extract were identified by comparing their calculated linear retention indices (IRLcal) and the linear retention indices-mass spectra (IRL-MS) present in the literature [18].

Acute Toxicity: Median Lethal Dose (LD50): Acute toxicity at single doses was evaluated according to Standard 423 given by the Organization for Economic Cooperation and Development (OECD), where the protocol used was the limit dose test up to 2000 mg/kg of the 1:1 mixture of extracts, in mice [19], observed for 14 days. Changes in behavior and other parameters such as changes in body weight, food intake, motor activity, diarrhea, changes in eye and skin color and death of the experimental animals were evaluated.

Induction of Prostate Neoplasia: The method of Bosland and Prinsen [20] was used to assess the protective effect on prostatic neoplasia, we used Testosterone 100 mg/kg (T) + Cyproterone 50 mg/kg (C) + NMU 50 mg/kg once time. Male Holtzmann strain albino rats were used, weighing an average of 200 ±20g and obtained from the National Institute of Health (INS) in Lima, Peru. The rats were housed in large, ventilated cages and fed ad libitum for 5 days. The sample size formula was used to calculate the required number of animals by comparing the means of numerical variables while accounting for the statistical standard deviation (X1-X2): A total of fifty-six animals were tested, with the mean being 2.1, the standard deviation for both groups being 1.5 (d1 and d2), the confidence level being 95% and the power of the test being 80%. In contrast, 56 male albino rats of the Holtzmann strain were purchased from the INS, with an average weight of 200±20 g. These were taken to the UNMSM's Biotherium and Pharmacology Laboratory in the Faculty of Human Medicine and housed there for seven days with free access to food and water (provided by the UNMSM's partnership with the Universidad Agraria La Molina in Lima, Peru). The rats were kept in a controlled environment with a temperature of 21±2 °C and a light/dark cycle of twelve hours.

After the rats had adjusted to their new environment, they were split into seven groups of eight using the following experimental design: (1) Negative control: physiological saline SSF 2 mL/kg (SSF); 2. Positive control (TCN): Testosterone 100 mg/kg (T) + Cyproterone 50 mg/kg (C) + NMU 50 mg/kg (N); 3) TNC-250 ChS supplemented with 250 mg/kg *Chuquiraga spinosa*; 4) TNC-100SrR supplemented with 100 mg/kg *Senecio rhizomatus* Rusby; 5) TNC-50ChS-50SrR supplemented with 50 mg/kg ChS and 50 mg/kg SrR; 6) TNC-250-ChS-100 SrR supplemented with 250 mg/kg ChS and 100 mg/kg SrR; 7) TNC-500ChS-200 SrR supplemented with 500mg/kg ChS and SrR 200mg/kg SrR. Cyproterone acetate was injected intraperitoneally (IPV) once daily (50 mg/kg body weight in olive oil) for eighteen days; testosterone enanthate was injected subcutaneously (SCV) once daily (100 mg/kg body weight) for three days; and a single dose of 50 mg/kg body weight (IPV) of N-methyl-nitrosourea (NMU) was administered via intraperitoneal route. The rats' weights were recorded after the conclusion of the experiment. The animals were weighed once a week and observed every day. Monthly weights are depicted in Figure 1. After a 12-hour fast at the end of the study period, blood samples were taken under ethyl ether anesthesia to assess biochemical and hematological

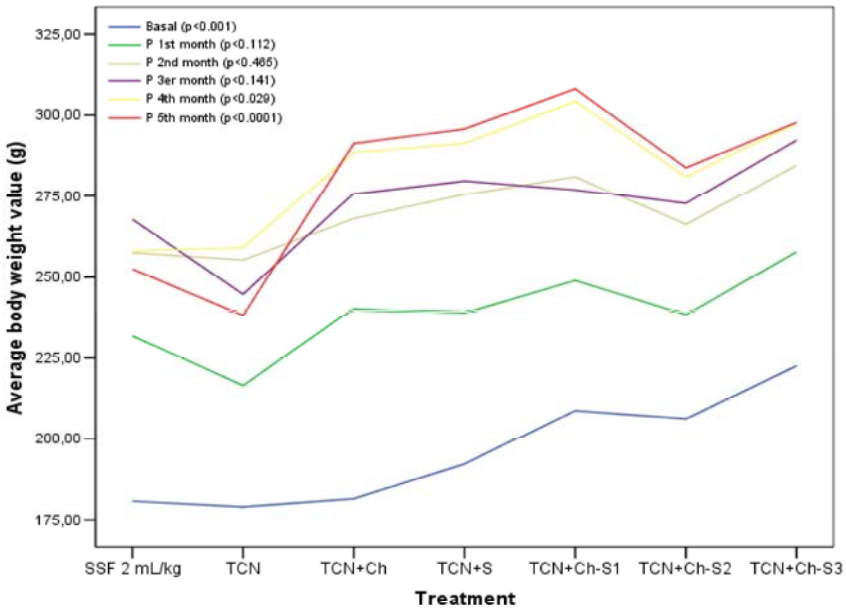

Fig. 1: Time course of body weight of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

indicators and the animals were subsequently euthanized with an overdose of pentobarbital (100 mg/kg). The prostates were dissected, cleaned in a physiological solution and measured using a vernier scale and a balance accurate to the nearest 0.001 gramme [21].

Hematological and Biochemical Parameters: Rats were given anesthesia with 30 mg/kg of pentobarbital before having their hearts punctured to draw blood. Hemoglobin concentration was measured spectrophotometrically. Both hematocrit and total leukocyte count was measured in a Neubauer chamber. Nitric oxide was assayed using the Griess reagent method [22] and malondialdehyde (MDA) concentration was determined by measuring the formation of a chromogenic MDA-TBA adduct following its reaction with 2-thiobarbituric acid (TBA) in blood serum [21]. The kinetic approach established by Jones *et al*. [23] was used to measure the activity of Glutathione peroxidase (GSH) in hemolyzed blood to estimate its antioxidant capacity. Spectrophotometric monitoring of NADPH oxidation at 340 nm and 37°C was performed. The amount of catalase in a sample was calculated by measuring the rate at which hydrogen peroxide is depleted from a sample using spectrophotometry at a wavelength of 240 nm and a temperature of 25°C [24] according to Claiborne method [25].

Prostate specific antigen (PSA) was assayed using commercial ELISA kit (add the information of the manufacturer, the country of origin), the sensitivity of the assay and the intra and inter assay precessions or coefficient of variation [5].

Prostate Ultrasound: The day before the animals were slaughtered, Doppler images were taken of the prostate in the different treatment (Chison D600 Vet, Jiang Su, China).

Histopathological Evaluation: The technique used by Arroyo *et al.* [5] was followed; in addition, the Gleason scale was used to measure the degree of aggressiveness of a prostate cancer, based on microscopic observation of the characteristics of the cells.

Statistical Analysis: Hematological and blood biochemistry study parameters such as body weight, hemoglobin, leukocytes, platelets, glucose, total cholesterol, high-density lipoproteins; triglycerides, alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT), nitric Oxide (NO), prostate specific antigen (PSA), were expressed as mean ± standard error. Homogeneity of variance was determined by Levene's test and normality by

Wilk-Shapiro W statistics. Data were analyzed using IBM SPSS v. 21 software. Replace SD by SEM in all the results section for the Care and Use of Laboratory Animals [26] and the current regulations of the Animal Welfare Act [27] were considered.

The present research was evaluated by the Ethics Committee of the Faculty of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos, through certificate No. 003-CE-UDI-FFB-2020 (Of. No. 004/FFB-UDI-2020 of 09-03-2020).

Rats were euthanized by intraperitoneal administration of pentobarbital at 100 mg/kg [28].

RESULTS AND DISCUSSION

Preparation of Ethanolic Extracts: From the aerial parts of *Chuquiraga spinosa* Less (Huamanpinta) and *Senecio rhizomatus* Rusby (Llancahuasi), a dry residue with stable weight was obtained and preserved in amber colored bottles with lids and refrigerated at 4ºC, for later study, after resuspension with a 3% solution of sodium polysorbate 80.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis Method: Table 1 shows the main metabolites observed by gas chromatography analysis, predominantly: 3,5-Cyclostigmast-22-ene, 6-methoxy, 1,4-Benzenediol and mono-tetradecyl ether.

Acute Toxicity: Median Lethal Dose (LD50). The single dose acute toxicity study evaluated according to OECD Standard 423, using the limit dose up to 2000 mg/kg in mice, in the 14 days of observation, showed no changes in behavior and other parameters such as alteration in body weight, food intake, motor activity, tremor, diarrhea, changes in eye and skin colors. Neither was death recorded in any of the experimental animals.

Experimental Animals: The increase in body weight of rats with induction of prostatic neoplasia was lower in the positive control group, that received only TCN (Testosterone 100 mg/kg, Cyproterone 50 mg/kg and NMU 50 mg/kg), as opposed to the other animals during the whole study (Fig. 1).

Induction of Prostate Neoplasia:

Histopathological Evaluation: The interpretation is described in Table 3.

Prostate cancer can be induced by NMU, as well as testosterone and cyproterone. Cyproterone, an antagonist of luteinizing hormone release, was administered orally to the animals in this study; this hormone's effects include a decrease in testicular androgens and the atrophy of prostatic epithelial cells. Testosterone propionate was administered subcutaneously to stimulate prostatic epithelial cell proliferation to its maximum extent and NMU was used to increase epithelial volume [5].

According to Table 1, a combination of 96% ethanolic extracts of *Chuquiraga spinosa* and *Senecio rhizomatus* contains 3,5-Cyclostigmast, a derivative of brassinosteroids; a previous study found that brassinosteroids extracted from Aeglemarmelos leaves reduced chromosome aberration frequency by modulating gene, nucleic acid and protein expression, suggesting a potential mechanism for this effect [29]. Since auxins, cytokinins, gibberellins, ethylene, and abscisic acid make up the other five classes of plant hormones, or phytohormones, brassinosteroids are often referred to as the "phytohormones of the 21st century" due to their extensive involvement in numerous physiological processes, including the regulation of growth and development in plants and fruits [30].

By inhibiting the growth of cancer cells more effectively than cisplatin, brassinosteroids demonstrate antitumor activity on the human lung carcinoma (A549)

Table 1: Main metabolites observed in the analysis by gas chromatography coupled to mass spectrometry (GC-MS) of the 1:1 Huamanpinta and Llancahuasi Mixture in acetone.

AreaPct	Library/ID
6.04	Phenol, 2,4-bis (1,1-dimethylethyl)-
2.20	Hexadecanoicacid
7.22	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl nonyl ester
38.02	3,5-Cyclostigmast-22-ene, 6-methoxy-, (3á,5à,6á,22E)-, (3á,5à,6á,22E)-
4.98	Phenol, 2,4-bis(1,1-dimethylethyl)-
3.11	Totarol<trans->, methylether
1.08	Totarolacetate<trans->Totarolacetate
22.35	1,4-Benzenediol, mono-tetradecylether
10.30	1,1'-(1,1'-Cyclopropylidenediethylidene)disemicarbazide
4.70	UNIDENTIFIED
100.00	

Table 2: Hematological parameters of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU

Treatment	Hb (g/dL)	Hcto (%)	Leukocytes/mm 3	Lymphocytes (%)	Platelets/mm 3
SSF 2 mL/kg	13.4±0.4	36.9±1.2	6525.0±1456.9	71.3±4.0	451.8±50.1
TCN	11.7±2.3	32.0±5.8	7350.0±1090.9	61.0±1.8	368.8±63.1
TCN+Ch250	13.6±1.2	37.1±2.5	8825.0±1117.7	67.3±5.9	480.5±37.1
TCN+S100	13.2±0.5	35.9±1.4	7850.0±2323.1	66.5±2.4	527.8±93.7
TCN+Ch-S1	13.4±0.9	36.6±2.6	7325.0±1075.1	68.0±6.2	476.3±29.1
TCN+Ch+S2	12.5±0.7	35.0±1.2	7000.0±2843.7	64.0±3.6	370.3±111.3
TCN+Ch-S3	12.8±0.6	35.4±1.3	7650.0±1941.6	62.0±1.6	511.5±61.3

SSF 2 mL/kg = normal or negative control with saline 2 mL/kg;
TCN = positive control Testosterone 100 mg/kg and Cyproterone 50 mg/kg and NMU 50 mg/kg; TCN+Ch250 = TCN and *Chuquiraga spinosa* 250 mg/kg;
TCN+S100 = TCN and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S1 = TCN and *Chuquiraga spinosa* 50 mg/kg and *Senecio rhizomatus* 50 mg/kg;
TCN+Ch-S2 = TCN and *Chuquiraga spinosa* 250 mg/kg and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S3 = *Chuquiraga spinosa* 500 mg/kg and *Senecio rhizomatus* 200 mg/kg.

Table 3: Histopathological interpretation of prostate in rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU

Treatment	Description	Conclusion
SSF 2 mL/kg	With physiological saline solution 2 mL/kg	Normal alveolar cells
TCN	Inducer Cyproterone + Testosterone + NMU (I) + saline 2 mL/kg diffuse alveolar enlargement, growth pattern basal cells pseudostratified stratum ciliare, cells retain nuclear size, structure is lost, no cellular atypia. Large number of intralveolar macrophages. Fairly extended growth pattern with very deep folds, high structural atypia, no stroma, no mitosis, mild focal cellular atypia, no aberrant mitosis, obvious architectural atypia. May go up to Gleason 7, but no mitosis.	Because of the growth pattern, a neoplasm has been reached, as it has exceeded grade 3, a Gleason 7 neoplasm pattern has been reached.
TCN+Ch250	Inductor + Humanpinta (250 mg/kg) areas of edema with stromal growth, basal layer is observed, focally a somewhat large nucleus with isonucleosis is observed. Basal layer and main cell layer are observed. Slight growth of internal folds. Union of folds in one of the foci. Stromal growth with visible blood vessels. Slight architectural alteration, no cellular alteration is observed. Union of the folds in three foci, but preserving the stroma.	A mild structural alteration without cellular atypia. Benign prostatic hyperplasia
TCN+S100	Large amount of fluid and macrophages, with presence of pseudostromata. Cribriform growth, stroma is still observed. Stroma in the folds and presence of blood vessels.	Architectural atypia with gleason 4 growth pattern.
TNC+Ch-S1	Inductor + Huamanpinta 50 mg/kg +Llancahuasi 50 mg/kg Glandular growth with abundant stroma and alveolar growth with folds not exceeding 20%. Pudostratified growth in its epithelium. Patterns with growth and junction of folds exceeding 50% of the diameter presenting stroma. There is cribriform growth. Scarce stroma in the epithelium and greater number of internal folds reaching up to 40% of its diameter. Pseudo-stratification and reptation	Gleason 3 and gleason 5 growth patterns
TNC+Ch-S2	Inductor + Huamanpinta 250 mg/kg +Llancahuasi 100 mg/kg presents architectural atypia without cellular atypia with folds up to 80% and alveolar septation. Growth of folds up to 50% with hyperchromasia.	Benign hyperplasia with cribriform pattern foci with a gleason 4
TCN+Ch-S3	Inductor + Huamanpinta 500 mg/kg +Llancahuasi 200 mg/kg abundant stroma is observed between the glands. Growth of internal folds reaching 40% and 30% of the diameter with stroma between folds. Abundant prostatic liquor with macrophages. Scarce stroma.	Architectural atypia, with focus of cellular atypia, Gleason 5

C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

and human hepatocellular carcinoma (Hep G2) tumour cell lines [31]. This suggests that combinations of classical antitumor drugs, such as cisplatin, with brassinosteroids may be beneficial.

Figure 1 and Table 2 reveal that the weight gain in the Huamanpinta and Llancahuasi extract group is accompanied by similar haematological values, indicating that there is no harmful effect at the levels tested. Increased GSH and CAT levels, in addition to decreased NO levels, demonstrate the extract groups' antioxidant ability when compared to the inductor group. When combined with SrR, the ChS 250 extract reduces prostate volume in comparison to the inductor.

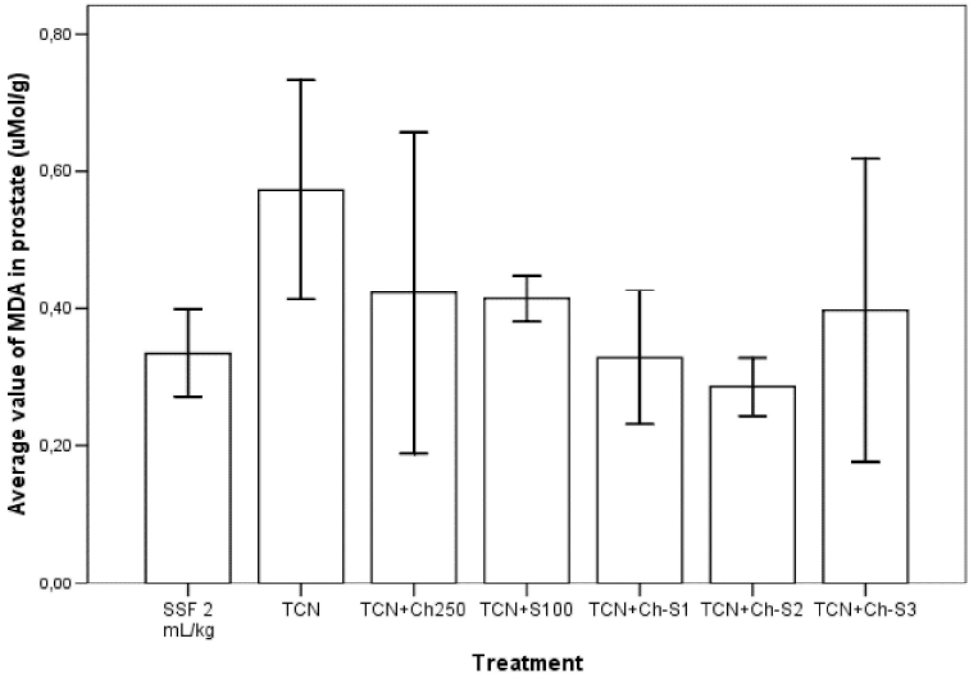

Fig. 2: Malondialdehyde (MDA) level in rat prostate with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

Apoptosis, cell cycle arrest and inhibition of cell migration are just some of the selective anticancer actions that the diterpene totarol has been shown to have in SGC-7901 human gastric cancer cells [32]. In addition to reducing the expression of penicillin binding protein 2a, a protein involved in the penicillin resistance of MRSA, totarol is a highly hydrophobic, diterpenoid product with a high phospholipid/water partition coefficient that can interfere with the structural integrity of the bacterial membrane, resulting in cell lysis. Recent studies reveal that totarol possesses anticancer, antioxidant and anti-inflammatory activity [14,33] and it has the potential to be used in clinical therapy and the prevention of food spoilage due to its ability to block the hemolytic proteins and enterotoxins generated by S. aureus.

Table 1 shows that 1,2,4-benzenetricarboxylic acid, 1,2-dimethyl nonyl ester, contributes antioxidant, antimicrobial and anticarcinogenic activity [34, 35]. The presence of hexadecanoic acid also provides antioxidant, hypocholesterolemic and anti-inflammatory effects.

In Figure 2, we can see that MDA levels have dropped. Malondialdehyde is a naturally occurring biomarker used to quantify oxidative stress [36]. It is produced when reactive oxygen species degrade polyunsaturated lipids, resulting in the formation of a reactive aldehyde that is toxic to cells. Figure 4 depicts changes in GSH, which may be explained by the fact that tumour cells produce elevated levels of oxygenated water (2OH2), which promotes tissue damage and mutation and thus aids in the growth, invasion and metastasis of tumor cells. Proliferation rates are also affected by intracellular GSH levels. Increased peroxide production, ROS levels and GSH oxidation are all associated with tumor cell proliferation [37].

The Gleason scale, created in the 1960s by pathologist Donald Gleason, is still used today to predict how aggressively prostate cancer will grow and spread among patients [38]. Gleason grades below 5 are disregarded. Low-grade cancer has a Gleason score of 6, the lowest possible score. Mid-grade cancers have a Gleason score of 7, while high-grade cancers have a score of 8 or 9. Cancers of a lower grade tend to progress more slowly and are less likely to metastasize [39,40]. In the current study, animals exposed just to the inducing toxicant had Gleason scores of 7, indicating significant malignancy, but animals treated with the compounds had scores that were lower (Table 2). There was a synergistic effect between a reduced Gleason score and the antioxidant effect across treatments, the data showed.

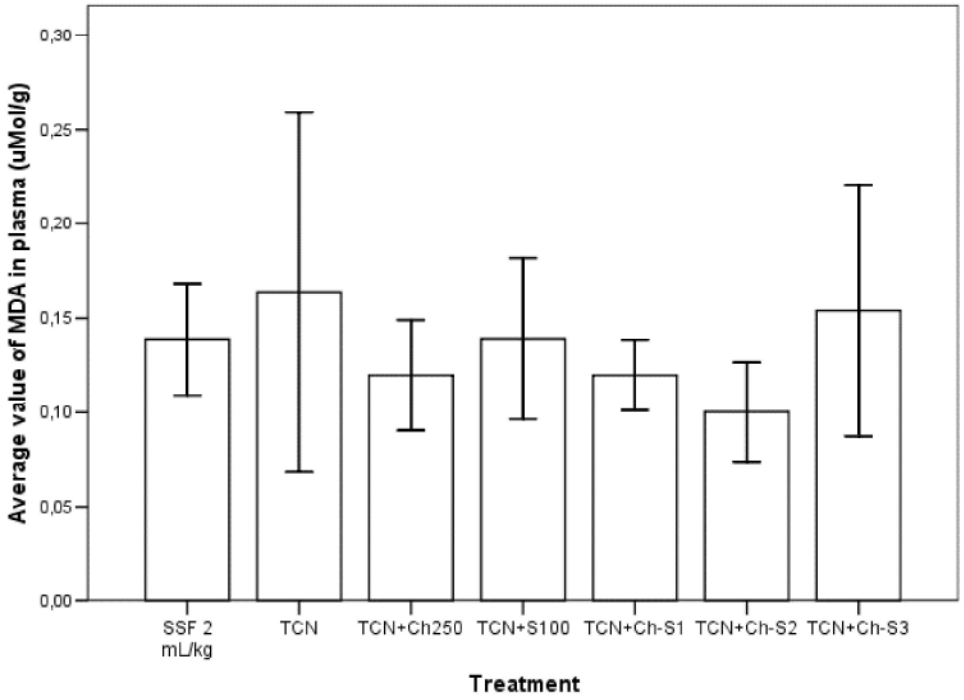

Fig. 3: Malondialdehyde (MDA) level in plasma of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

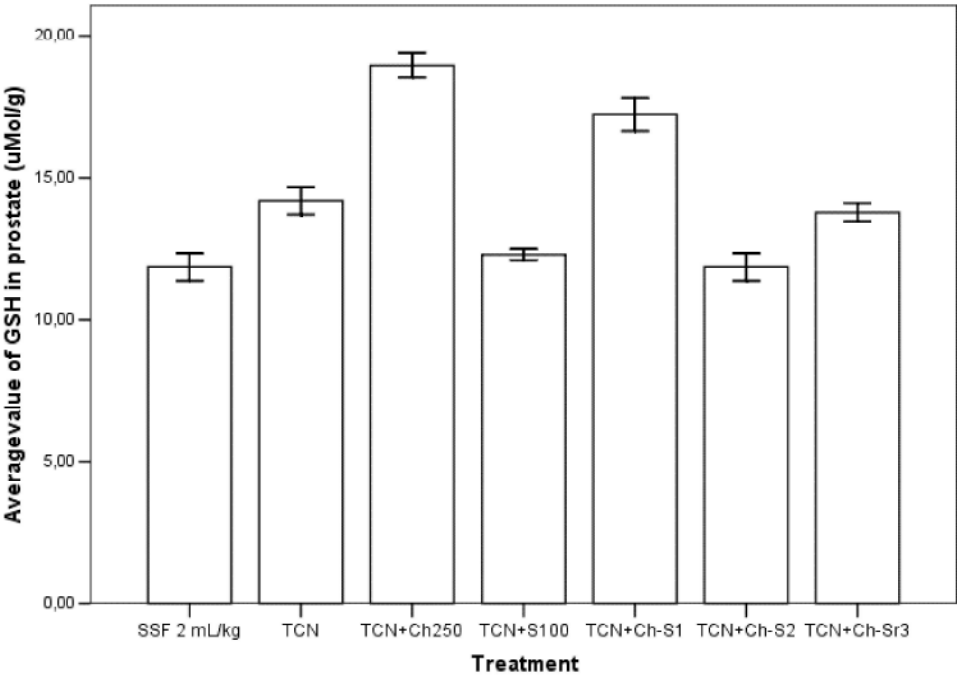

Fig. 4: Reduced glutathione (GSH) level in prostate of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

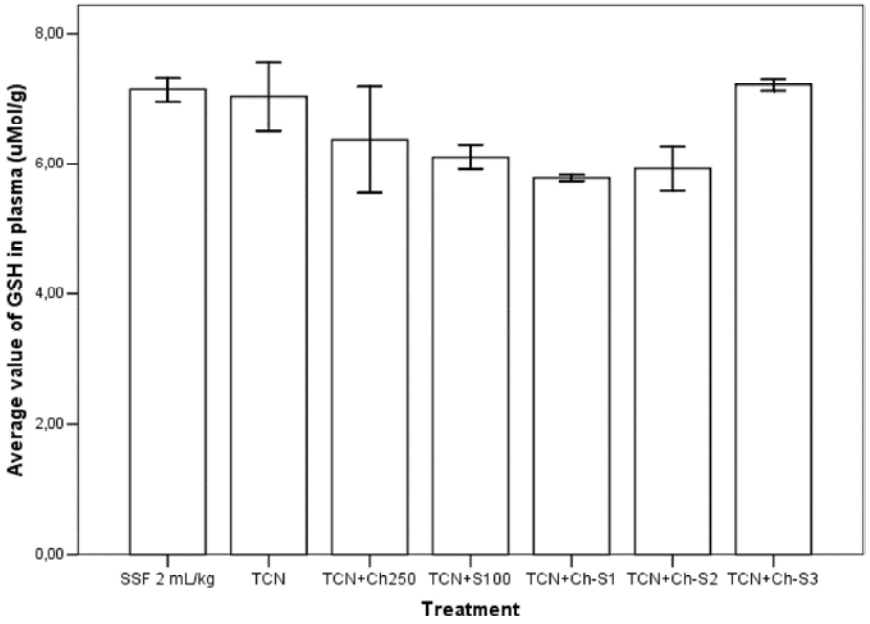

Fig. 5: Reduced glutathione (GSH) level in plasma of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
SSF 2 mL/kg = normal or negative control with saline 2 mL/kg;
TCN = positive control Testosterone 100 mg/kg and Cyproterone 50 mg/kg and NMU 50 mg/kg; TCN+Ch250 = TCN and *Chuquiraga spinosa* 250 mg/kg;
TCN+S100 = TNC and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S1 = TCN and *Chuquiraga spinosa* 50 mg/kg and *Senecio rhizomatus* 50 mg/kg;
TCN+Ch-S2 = TCN and *Chuquiraga spinosa* 250 mg/kg and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S3 = *Chuquiraga spinosa* 500 mg/kg and *Senecio rhizomatus* 200 mg/kg.

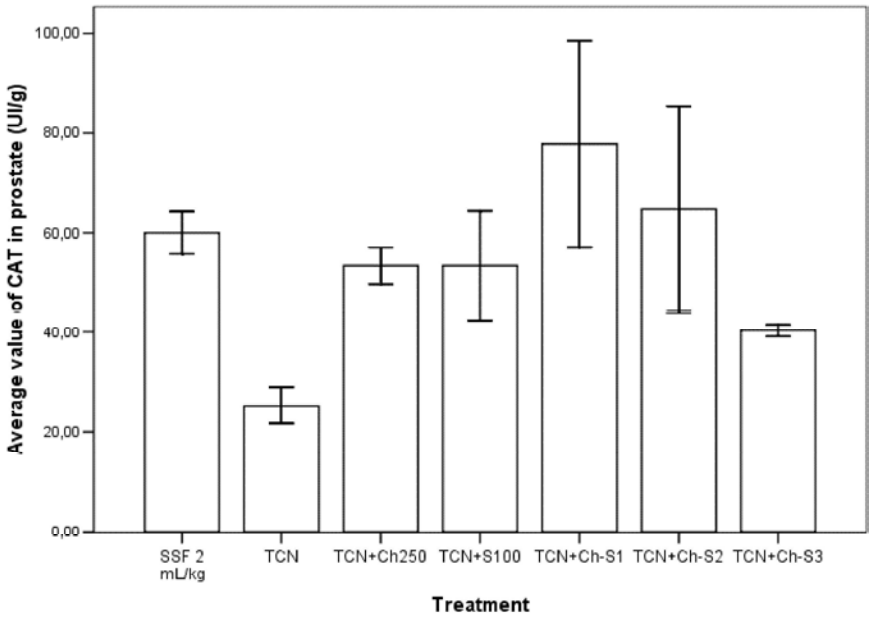

Fig. 6: Catalase (CAT) level in prostate with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU. C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

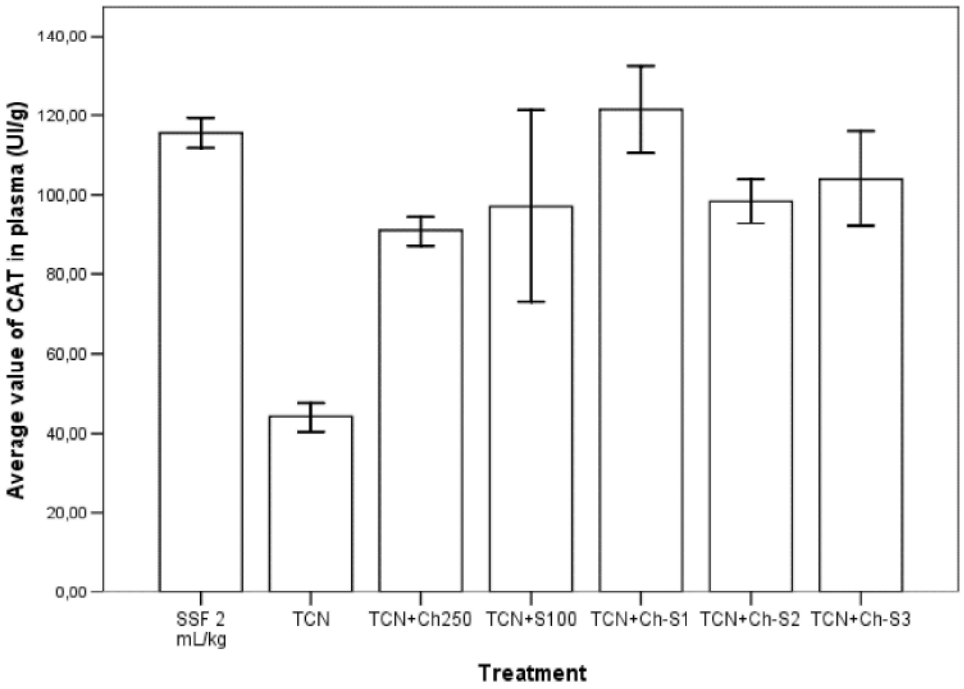

Fig. 7: Catalase (CAT) level in plasma d with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU. C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

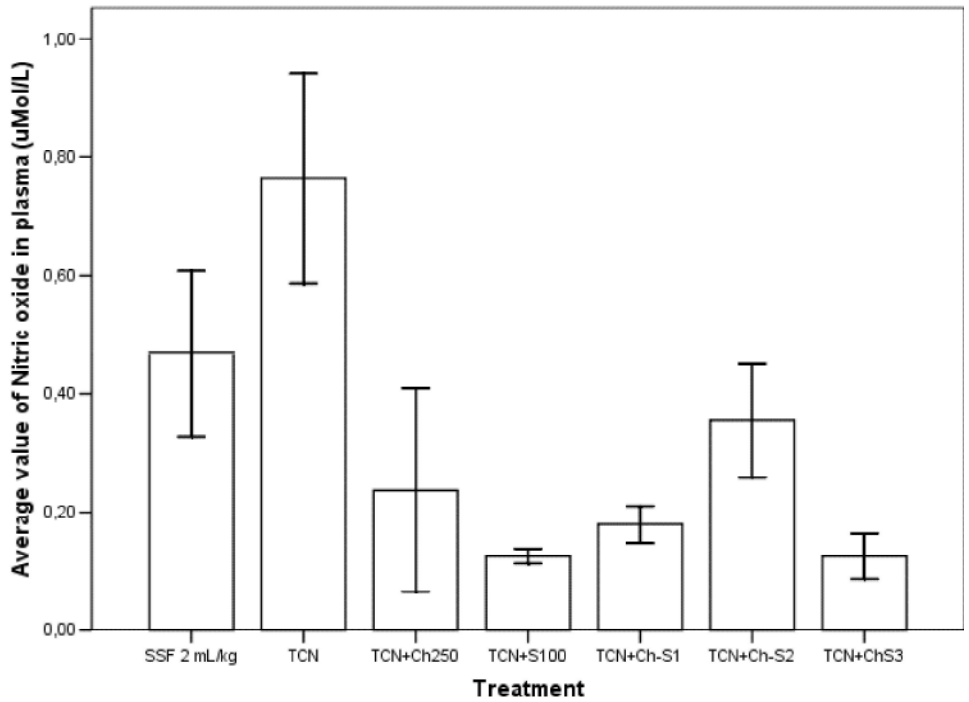

Fig. 8: Blood nitric oxide level with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU. C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

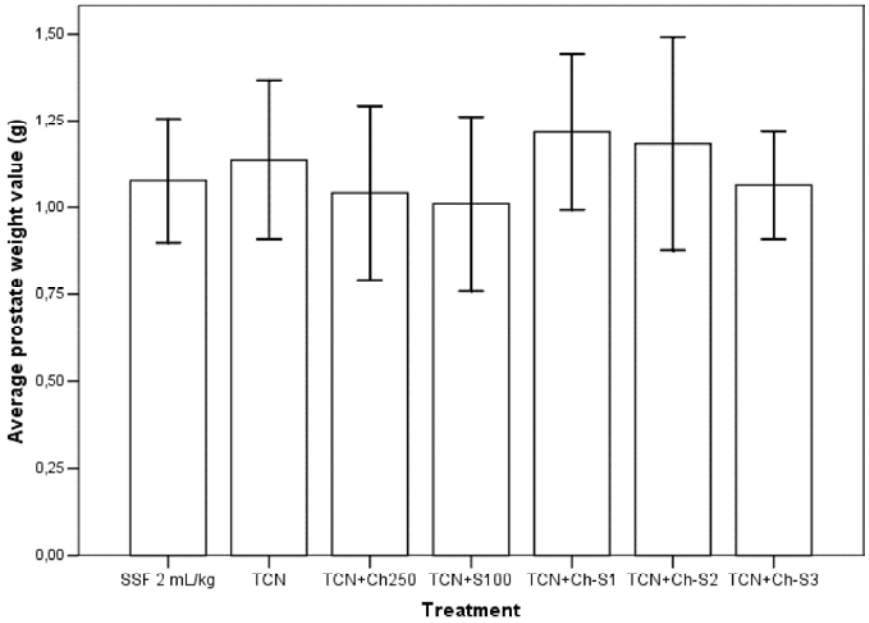

Fig. 9: Prostate weight (g) with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

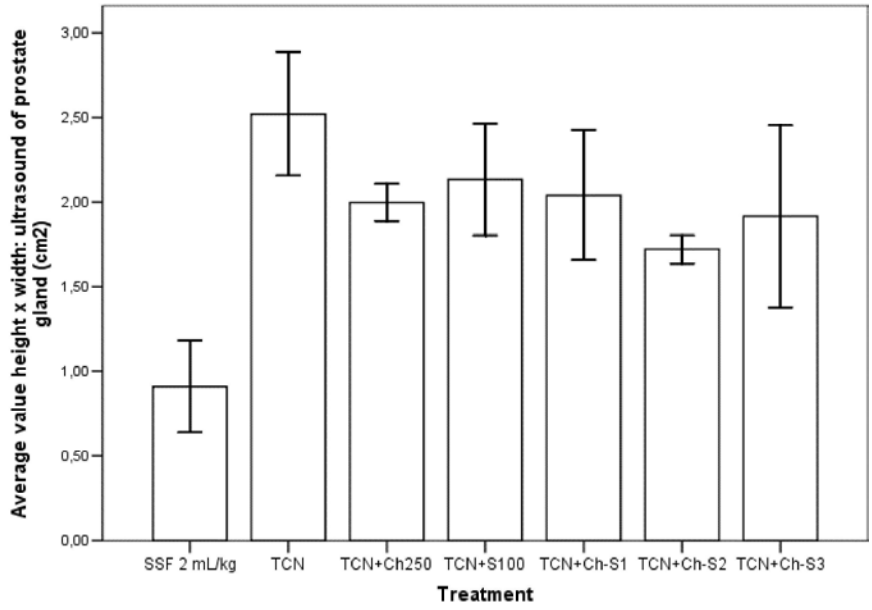

Fig. 10: Ultrasound dimension of prostatic height X width (cm^2) with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

Research into the identification and isolation of larger quantities of metabolites is encouraged so that, in combination with studies of bibliographic review, this information can be used to explain potential molecular pathways. To further clarify the mechanism of action of the products used, safety and efficacy studies on

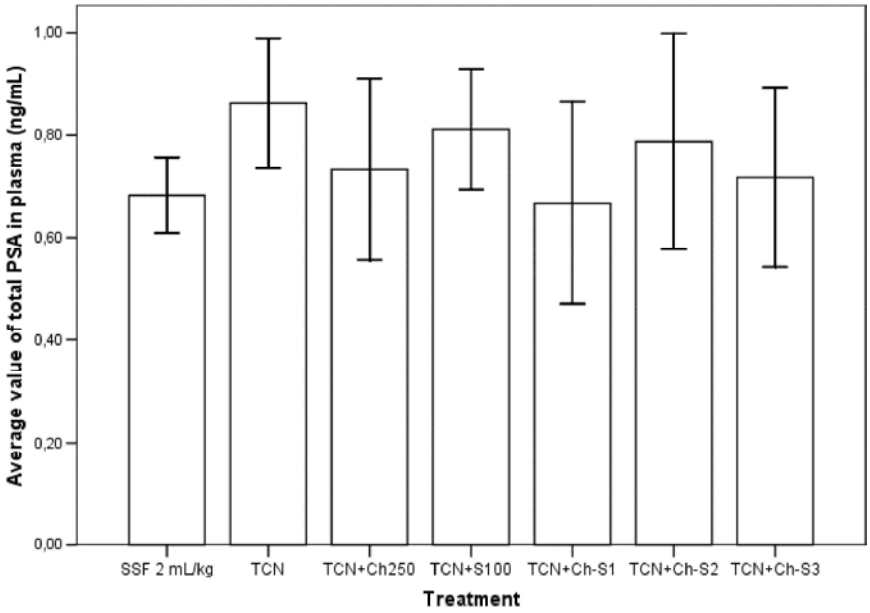

Fig. 11: Total prostatic antigen level in plasma of rats with induction of prostatic neoplasia by Testosterone, Cyproterone and NMU.
C-ve (SSF); TCN (C+ve); TCN-Ch250, TCN+S100 (TNC-100 Sr); TCN+Ch-S1(TCN -Ch 50 -Sr 50); TCN+Ch-S2 (TCN -Ch250 -Sr100);TCN+Ch-S3 (TCN-Ch 500 -Sr 200).

prostatic neoplasia should also be conducted to find an adequate association to continue the search for a mean effective dose and to potentially conduct genetic studies.

The ethanolic extract of *Chuquiraga spinosa* Less (Huamanpinta) in combination with *Senecio rhizomatus* Rusby (Llancahuasi) has been shown to have a chemopreventive impact on prostate neoplasia produced in rats. The phytochemical study has revealed secondary metabolites in higher concentration when associating ethanolic extracts of the aerial parts of *Chuquiraga spinosa* Less and *Senecio rhizomatus* Rusby: 3,5-Cyclostigmast-22-ene, 6-methoxy-, (3á,5à,6á,22E)-; 1,4-Benzenediol, monotetradecylether;1,1'(1,1' Cyclopropylidenediethylidene) disemicarbazide, followed by totarol, among others. The administration of an ethanolic extract of the aerial portions of *Chuquiraga spinosa* Less in conjunction with *Senecio rhizomatus* Rusby has been shown to have a positive effect on antioxidant activity and oxidative stress indicators in both vitro and in vivo. When the ethanolic extract of the aerial parts of *Chuquiraga spinosa* Less associated to *Senecio rhizomatus* Rusby was given to rats with induced prostate neoplasia, the levels of haematological and biochemical parameters evaluated were observed to be within the allowed values and the anatomopathology did not show morphological changes attributed to toxicity. In rats where prostate neoplasia was induced, a synergistic protective effect was identified; however, the response generated by the association was dose-independent [41,42].

CONCLUSIONS

Hydroquinone, also known as 1,4-benzenediol, is present in the 1:1 Huamanpinta and Llancahuasi mixture, according to phytochemical analysis. This compound is thought to be responsible for the observed protective effect, as it inhibits the expression of proinflammatory cytokines like TNF-, IL-1, IL-2, IL-6 and IL-10 by suppressing Akt kinase in the NF-B pathway in In melanocytes, it prevents the production of melanin by inhibiting the enzymatic oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA). On the flip side, melanocytes' other metabolic functions are stifled. Bleached parts will repigment after being exposed to sunlight or UV rays [43]. Increased Bcl2 protein,

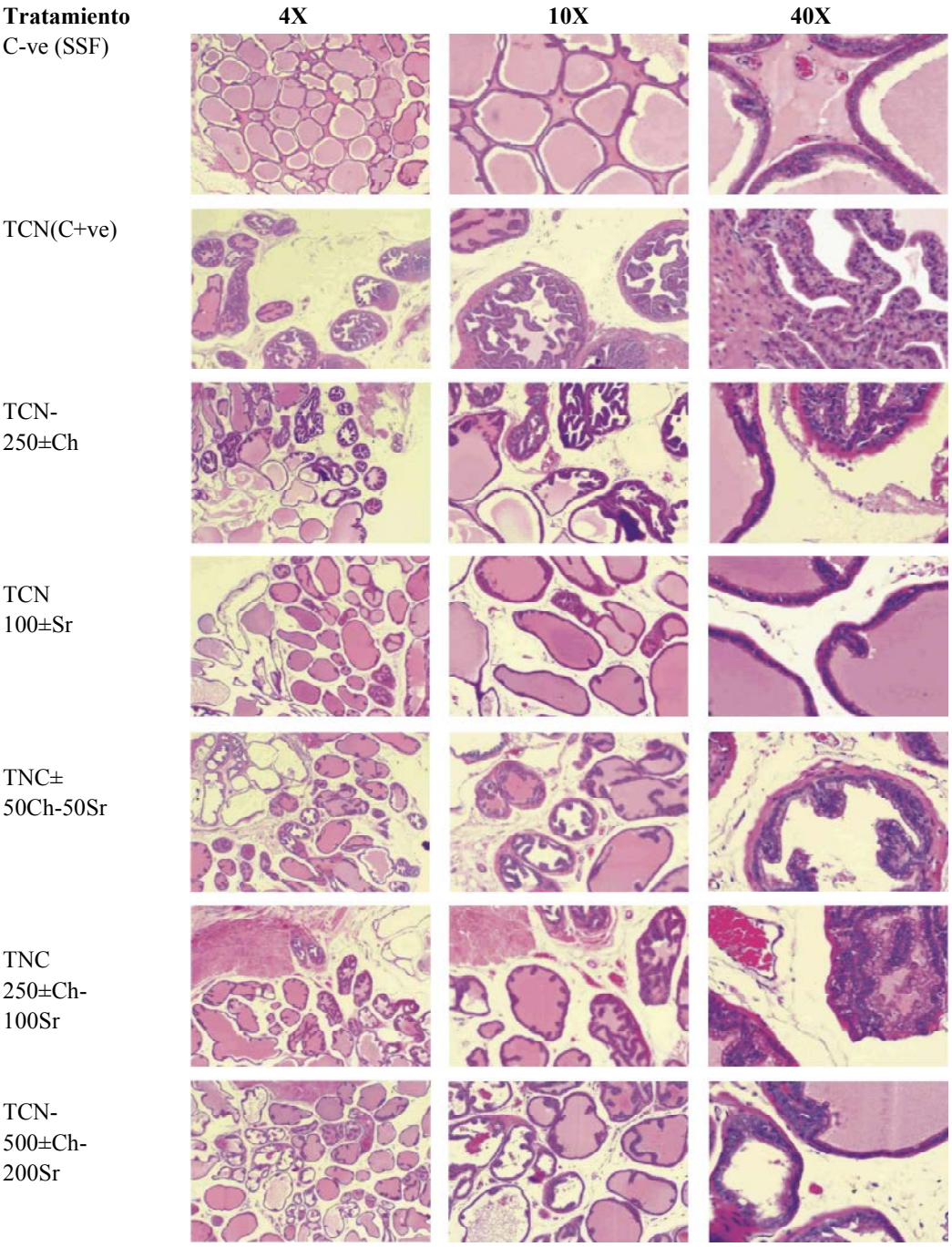

Fig. 12: Histopathological study of the prostate of rats with hyperplasia induction by Testosterone, Cyproterone and NMU.
SSF 2 mL/kg = normal or negative control with saline 2 mL/kg;
TCN = positive control Testosterone 100 mg/kg and Cyproterone 50 mg/kg and NMU 50 mg/kg; TCN+Ch250 = TCN and *Chuquiraga spinosa* 250 mg/kg;
TCN+S100 = TNC and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S1 = TCN and *Chuquiraga spinosa* 50 mg/kg and *Senecio rhizomatus* 50 mg/kg;
TCN+Ch-S2 = TCN and *Chuquiraga spinosa* 250 mg/kg and *Senecio rhizomatus* 100 mg/kg;
TCN+Ch-S3 = *Chuquiraga spinosa* 500 mg/kg and *Senecio rhizomatus* 200 mg/kg.

Fig. 13: *Chuquiraga spinosa* Less (Huamanpinta)

Fig. 14: *Senecio rhizomatus* Rusby (Llancahuasi)

mutagenesis, carcinogenesis, weakened DNA and its repair mechanisms and inhibition of apoptosis [44, 45] are all effects of hydroquinone and its metabolites, which also affect the overexpression of Bcl2, which blocks the activity of Bax. There is a need for more study into the anticancer effect of hydroquinone (HQ, 1,4-benzenediol), a hydroxylated benzene metabolite with antioxidant, neuroprotective, immunomodulatory and anti-inflammatory effects.

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