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EVALUATION OF THE ANTIOXIDANT EFFECT OF ALLIUM SATIVUM.L (GARLIC) FRESH JUICE EXTRACT ON A MODEL OF BEGNINE HYPERTROPHY OF THE PROSTATE IN BALB/C MICE

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ABSTRACT

Summary: Benign prostatic hyperplasia (BPH) or prostatic adenoma is a disorder characterized by enlargement of the prostate and obstructive symptoms. The aim of this study was to determine the phytochemical composition and anti-oxidative effect of fresh AS juice extract on a mouse model of BPH. **Materials and Methods:** Mice were divided into four groups: group I: control (0.2 ml/kg olive oil), group II: BPH (0.072 mg/kg TE), group III: preventive (BPH + 20 mg/kg AS + 0.072 mg/kg TE) and group IV: curative (BPH + 0.072 mg/k TE + 20 mg/kg AS). BPH was induced by intramuscular injections of testosterone enanthate (TE) and its action potentiated by the administration of b(a)p. At the end of the various treatments, the animals were sacrificed. The prostates were removed and weighed to determine the prostate weight index (PW). Serum levels of testosterone (free TE), prostate-specific antigen (free PSA) and the oxidative stress enzyme activities of malondialdehyde (MDA) and superoxide dismutase (SOD) were then determined using enzyme immunoassay techniques. Phytochemical studies were carried out to determine polyphenol and flavonoid content. **Results:** The results show a significant reduction in the prostate weight index (PW) in groups III and IV ($p<0.0024$), serum levels of free TE hormones in groups III ($p=0.0051$) and IV ($p=0.0253$), and free PSA in groups III ($p=0.0031$) and IV ($p=0.0289$) after treatment with fresh AS juice, compared with group II. Administration of fresh AS juice resulted in a significant dose-dependent decrease in cystolic MDA levels in groups III ($p=0.004$) and IV ($p=0.012$). And a significant dose-dependent increase in the cystolic level of SOD in groups III ($p=0.0001$) and IV ($p= 0.0077$). Phytochemical analysis of fresh AS juice revealed the presence of tannins, flavonoids and reducing compounds. The highest levels of polyphenols and flavonoids were observed in the stock solution (C1=20mg/mL), i.e. 588mgEAG/100gMS in polyphenol and 8.10mgERu/100gMS in flavonoids. **Conclusion:** Fresh AS juice, administered to animals either as a preventive or curative measure, attenuates the development of BPH by regulating oxidative stress in the prostate.

KEYWORDS: *Allium sativum. L*, phytochemicals, benign prostatic hyperplasia, oxidative stress.

INTRODUCTION

Benign prostatic hyperplasia (BPH) or prostatic adenoma is a disorder characterised by prostate enlargement and obstructive symptoms.[1] It is caused by excessive but non-malignant growth of prostatic stromal and epithelial cells.[2] The prevalence of BPH increases with age and continues to rise as the population ages.[3] It is estimated that 50% of men over the age of 50 and 90% of men at the age of 80 will develop BPH.[4]

Oxidative stress is one of the factors implicated in the development of prostate disease.[5] It is a situation in which the oxidative and antioxidant systems are out of balance with an overproduction of reactive oxygen species (ROS) and a comparative deficiency of antioxidants.[6] And excessive ROS accumulation can induce DNA damage, lipid peroxidation and protein modification, subsequently leading to cellular dysfunction and tissue damage.[5] Clinical and experimental studies have confirmed that oxidative stress is a key contributor to the pathogenesis of BPH.[7]

Several studies have addressed the relationship between the development of BPH and oxidative stress.

Allium sativum.L (AS) is a medicinal plant whose pharmacological effects are attributed to several bioactive substances such as vitamins C and E, β-carotene and polyphenols, flavonoids.[8] The antioxidant properties of AS have been the subject of several studies. These substances are capable of preventing the formation of free radicals.[9] Studies have shown that allicin, the main compound in AS, increases serum levels of catalase and glutathione peroxidase, two very powerful antioxidant enzymes.[10] The aim of this study was to determine the phytochemical composition and the preventive and curative properties of AS fresh juice extract on BPH induced in mice by enanthate testosterone (TE), potentiated by benzo(a)pyren (b(a)p).

MATERIALS AND METHODS

Chemicals and reagents

Standard laboratory grade chemicals and reagents were used in this study. The enanthate testosterone (TE) solution marketed under the name Androtardyl® was used. Benzo (a) pyrene was sourced from Shejiang Province (China). ELISA kits for free testosterone (free TE), prostate specific antigen (free PSA), malondialdehyde (MDA) and superoxide dismutase (SOD) were supplied by SUNLONG BIOTECH CO. LTD, China.

Plant material and Extraction of fresh juice

Fresh Allium sativum bulbs were obtained from markets in Brazzaville. Extraction was carried out using 500.24 g of garlic bulbs (Corresponding to 12 cloves), crushed using a mortar and pestle. The crushed material was pressed and the juice filtered, then stored at +4°C. This extraction yielded 120 ml of garlic juice, giving a yield of 4.16%.

Phytochemical analysis

Chemical tube tests were used to identify the main chemical families present in AS fresh juice extract (Alkaloids, tannins, flavonoids, reducing sugars and saponosides).

Identification of alkaloids

5 ml of AS aqueous extract was placed in a test tube. Then 1 ml of 1N hydrochloric acid and a few drops of reagents were added. The formation of a red precipitate (with Dragendorff's reagent) or a yellowish precipitate (with Mayer's reagent) indicated the presence of alkaloids. **[11]**

Identification of tannins

Tannins were identified by mixing 5 ml of aqueous AS decoction with 1 ml of an aqueous iron chloride solution. In the presence of tannins, a greenish or blue-blackish colour develops.[12]

Identification of flavonoids

5 ml of 5% AS decoction, 5 ml of a hydrochloric acid (HCL) solution and 1 ml of iso-amyl alcohol and some magnesium chips were mixed. A change in colour indicated the presence of the following compounds.[11]

– Orange-yellow, for flavones ;
– Purplish pink, for flavanones ;
– Red for flavonols and flavonols.

Identification of reducing sugars

To 5 ml of AS decoction was added 1 ml of Fehling's liqueur. The formation of a brick-red precipitate indicated the presence of reducing sugars.[11]

Identification of saponins

5 ml of aqueous extract of AS was mixed with 5 ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was considered an indication of the presence of saponins.[11]

Determination of total polyphenols (TPP)

The principle is based on determining the optical densities of the extracts in relation to those of a standard gallic acid solution of known concentration. Briefly, 0.1ml of SA extract was mixed with 0.9ml distilled water and 0.9ml Folin-Ciocalteu reagent (1N), followed by 0.2ml Na_2CO_3 solution (20%). This mixture was incubated at room temperature for 40 minutes, protected from light. The absorbance was then measured using a spectrophotometer at 725 nm against a methanol solution used as a blank. A calibration curve was determined using the standard solution of gallic acid under the same conditions as the samples to be analysed. The results obtained were expressed as mg gallic acid equivalent per gram of dry matter (mgEAG/gMs).[13]

Determination of total flavonoids

To 250 µl of extract and 1 ml of distilled water were added 75 µl of $NaNO_2$ solution (5%) and then 75 µl of $AlCl_3$ (10%), 5 minutes later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the mixture obtained was measured using a UV-visible spectrophotometer at 510 nm and the results were expressed as mg rutin equivalent per 100 grams of dry matter (mgERu/100g Ms).[12]

Animals and Treatment

Ten-week-old male Balb/c mice, weighing an average of 18 ± 2 g, from the animal house of the Faculty of Health Sciences at the University Marien Ngouabi (Brazzaville, Congo) were used. They were reared under standard conditions with free access to water and food.

All experiments were conducted in compliance with Directive 2010/6006/EU on the protection of laboratory animals.[14] Before the start of the experiment, the animals were acclimatised to laboratory conditions for 2 weeks. They were then randomly divided into four groups of 6 animals each and treated as follows

- Group I: control (0.2 ml/kg olive oil orally),
- Group II: BPH (2 mg/kg of b(a)p intraperitoneally and TE enanthate 0.72 mg/kg intramuscularly)
- Group III (preventive) and IV (curative): BPH+ AS juice extract 20 mg/kg.
  AS juice extract was administered to animals for 21 days in group III (preventive titre) and after in group VI (curative titre) BPH induction.[15]

Induction of benign prostatic hyperplasia

BPH was induced by intramuscular injections of TE enanthate dissolved in distilled water, at a dose of 0.72 mg/kg/mouse, four times a week for 21 days.[15] B(p)a, administered intraperitoneally at a dose of 2 mg/kg, 24 h prior to enanthate TE injection, potentiated the development of BPH.

Animal Sacrifice and Sampling

At the end of the experiment, the mice were sacrificed under diethyl ether anesthesia (Ether cooper®). Blood samples were taken in dry tubes from the retro-orbital sinus. The blood was centrifuged at 3,000 rpm for 15 minutes and the serum was aliquoted and stored at 4°C until biological analysis. The prostate glands were removed and immediately placed in PBS buffer for assay of hormonal parameters and oxidative stress.

Determination of the prostate index

After the animals were killed, the prostate was removed, rinsed with saline and immediately weighed. The prostate index (PW) was then calculated using the following formula:

$$\text{Prostate index} = \frac{\text{Prostate weight}}{\text{Body weight}} \times 100$$ [16]

Biological analyses

Assessment of hormones

Serum concentrations of testosterone (free TE) and prostate specific antigen (free PSA) were determined by ELISA, using commercial kits (free TE, free PSA ELISA kits) in accordance with the manufacturer's instructions.

Assessment of oxidative stress

Prostates collected in 1 ml of PBS buffer (0.1 M, pH 7.4) were individually ground using an applicator. The homogenate obtained was then centrifuged at 3,000 rpm for 25 minutes. The supernatant obtained was aliquoted and used to determine the enzymatic activities of malondialdehyde (MDA) and superoxide dismutase (SOD) by enzyme-linked immunosorbent assay, in accordance with the manufacturer's instructions.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Graph Pad Prism version 8 XML project software. Differences between the control and treated groups were analysed using Student's t-test and the significance threshold was set at $p < 0.05$.

RESULTS

Phytochemical analysis

Chemical screening

Phytochemical analysis of fresh AS juice revealed the presence of tannins, flavonoids and reducing compounds (Table I).

Table I: Phytochemical composition of fresh AS juice.

	Chemical families				
	Alkaloids	**Flavonoids**	**Tannins**	**Saponins**	**Tri-terpenes and Sterols**
Allium sativum. L	++	+++	++	-	++++

Legends:
(+++): Strongly present; (++): moderately present; (+): weakly present and (-): absent.

Quantitative determination of total polyphenols and flavonoids

The highest levels of polyphenols and flavonoids were observed in the stock solution (C1=20mg/mL), i.e. 588mgEAG/100gMS for polyphenols and 8.10mgERu/100gMS for flavonoids. These results are shown in Figure 1.

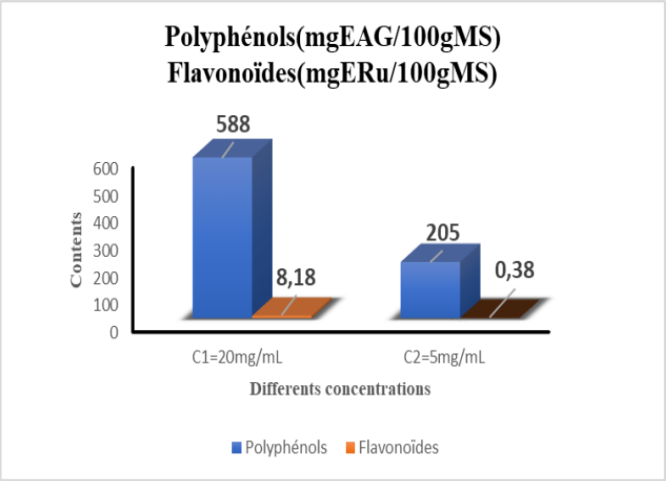

Figure 1: Polyphenol and Flavonoid content.

Effect of AS juice extract on prostate weight index

Table II, shows the weight and prostate weight index of animals after treatment with the different products. Analysis of these results shows a significant reduction in prostate weight index ($p<0.0024$) in animals treated with AS juice extract, compared with animals in group II (BPH).

Table II: Effects of AS juice extract administration on body weight and prostate weight index in animals.

Groups	Body weight (g)	Prostate weight	Prostate weight index
Group I (CTRL)	22,9 ±1,060	0,022±0,001	0,103±0,010
Group II (HBP)	29,4±1,020	0,058±0,002 [a]	0,230±0,008 [a]
Group III (preventive)	27,5±0,883	0,034±0,005 [b]	0,136±0,021 [b]
Group VI (curative)	27,7±0,748	0,035±0,008 [b]	0,141±0,032 [b]

Results are expressed as mean ± standard error. [(a)]: p= 0.0001 group I vs group II. [(b)]: p= 0.0024 group III and IV vs group II. n=06 animals per group.

Effects of AS juice extract on hormone levels

Effects on free testosterone (free TE) levels

Administration of AS juice extract caused a significant decrease in circulating free TE levels in animals in groups III (p=0.0051) and IV (p=0.0253), compared with animals in group II (BPH). These results are shown in figure 2.

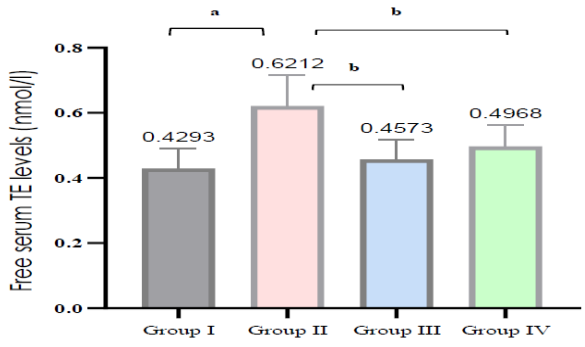

Figure 2: Effects of AS juice extract administration on free testosterone levels in animals.

Results are expressed as mean ± standard error. ([a]): p=0.0020 group I vs group II. ([b]): group II vs group III (p=0.0051) and group IV (p=0.0253). n=06 animals per group.

Effects on free PSA levels

Figure 3 shows the effects of AS juice extract on animals in the benign prostatic hypertrophy model. These results show a significant increase (p=0.0075) in PSA levels compared with Group I (control) animals. AS juice extract administered to the animals caused a significant decrease in free PSA levels in group III (p=0.0031) and

IV (p=0.0289) animals, compared with group II (BPH) animals. These results are shown in figure 3 below.

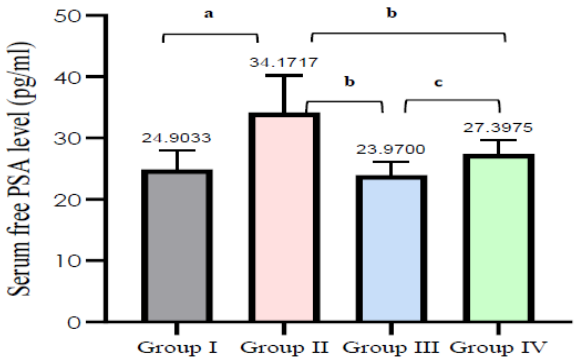

Figure 3: Effects of AS juice extract administration on free PSA levels in animals.

Results are expressed as mean ± standard error. ([a]): p=0.0075 group I vs group II; ([b]): group II vs group III (p=0.0031) and IV (p=0.0289); ([c]): p= 0.0241 group III vs group IV, n=06 animals per group.

Effect of AS juice extract on oxidative stress

Cystolic MDA oxidative enzyme activity

Figure 4 shows the effects of garlic juice on MDA enzyme activity in animals. Analysis of these results shows a significant decrease group III (p=0.004) and group IV (p=0.012) in MDA activity after treatment with AS juice, compared with the group II (HBP).

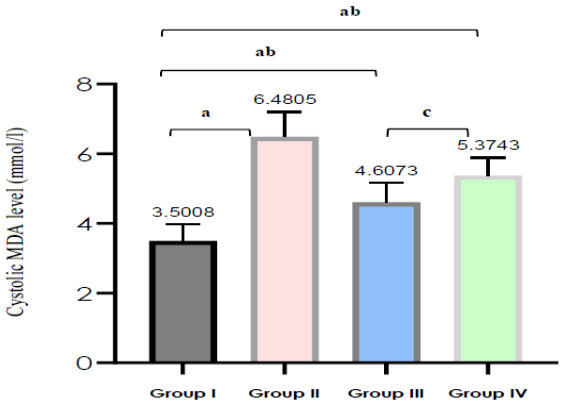

Figure 4: Effects of AS juice extract administration on cytosolic MDA activity in animals.

Results are expressed as mean ± standard error. ([a]): p=0.0001 group I vs group II. ([b]): group II vs group III (p=0.004) and IV (p=0.012). ([c]): group III vs group IV (p=0.0324). n=06 animals per group.

Cystolic SOD antioxidant enzyme activity

Figure 5 shows the effects of AS juice extract on SOD enzymatic activity in animals. Analysis of these results shows a significant increase group III (p=0.0001) and group IV (p=0.0077) in SOD activity after treatment with AS juice, compared with the group II (HBP).

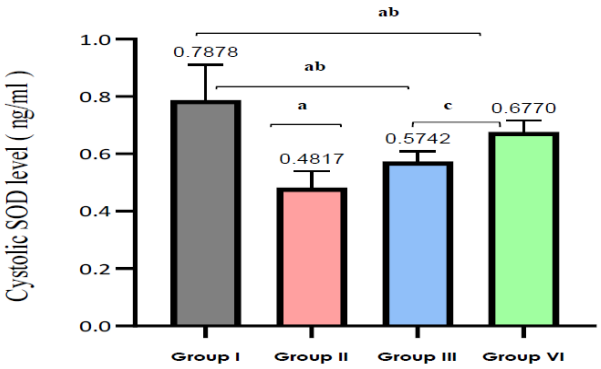

Figure 4: Effects of AS juice extract administration on cytosolic SOD activity in animals.

Results are expressed as mean ± standard error. ([a]): p=0.0001 group I vs group II. ([b]): group II vs group III (p=0.0001) and IV (p=0.0077). ([c]): group III vs group IV p=0.0008. n=06 animals per group.

DISCUSSION

Allium sativum.L (AS) is a plant of the alliaceae family containing several chemical compounds with antioxidant properties.[9] Our objective was to determine the phytochemical composition and the preventive and curative properties of the extract of fresh SA juice on the BPH model in vivo. BPH is a chronic andrological disease characterised by extensive testosterone-induced inflammation in epithelial cells and hyperplasia.[17] Clinically, BPH is characterised by heavy urination and lower urinary tract symptoms, affecting patients' quality of life. Numerous complications of BPH have been described, including urinary tract infections, acute urinary retention, urinary stones and renal failure.[18] Oxidative stress has been implicated in the development of BPH.[7]

Chemical screening revealed the presence of alkaloids, tannins, flavonoids and reducing sugars. However, we did not find any saponosides. Quantification of phenolic compounds (polyphenols and total flavonoids) in the extract was carried out using the aluminum trichloride (AlCl3) method. The results obtained show that the highest levels of polyphenols and flavonoids were observed in the solution (C1=20mg/mL), with a highly positive level of flavonoids recovered of 8.18 compared to 0.38 in the solution (C2=5mg/mL). These observations are in line with those made by other authors.[19,20]

In this study, we modelled BPH in mice by repeated administration of testosterone. The short-term effect of testosterone was potentiated by a single injection of B(a)P, as reported by Konishi N et al (1995).[21] The establishment and progression of hyperplasia was confirmed by increases in prostate index (PW), PSA levels and MDA and SOD enzymatic activities, as described in the literature.[22,23] Repeated administration of testosterone has been shown to induce prostate hyperplasia, resulting in an increase in prostate weight (and therefore prostate index) and an increase in markers of antioxidative stress.

Exogenous TE, is known to power on experimental models cell proliferation associated with inhibition of apoptosis.[24] This action of exogenous testosterone, potentiated by B(a)P in the present study had resulted in a significant increase in the prostate index. The results obtained in this study show that treatment with AS juice at a dose of 20 ml/kg (preventive or curative) resulted in a dose-dependent reduction in the prostate index. This effect of SA juice could be explained by an inhibition of the abnormal regulation of apoptosis caused by testosterone and B(a)P.[25] Biomarker analysis showed a significant reduction in prostate index, PSA levels and progression of prostatic hyperplasia in the extract groups compared with the disease group. Figures 2 and 3 also show a marked increase in free TE and free PSA levels in the treated groups. Indeed, injection of exogenous TE increases the level of intra-prostatic TE which produces a higher activity of the 5 α -reductase enzyme, leading to an accumulation of dihydrotestosterone (DHT). This accumulation in prostate tissue is sufficient to produce an increase in the androgen-dependent expression of growth factors. AS juice extract administered as a preventive and curative measure significantly reduced free PSA levels compared with animals in the BPH group. The prostate is one of the organs vulnerable to oxidative DNA damage due to faster cell turnover and the presence of fewer DNA repair enzymes.[26] In this study, induction of benign prostatic hyperplasia resulted in overexpression of MDA activity and decreased SOD compared to control animals, as reported by Z. Ouyang et al (2015).[27] Administration of AS juice extract, as a preventive or curative measure, improved intracellular enzyme activity, resulting in a significant decrease in MDA and a considerable increase in SOD. These two enzymes are important components of the antioxidant defence system.

Thus, the biological effects observed with fresh AS juice would be supported by the chemical composition of this extract, rich in flavonoids and phenolic compounds known for their anti-tumour, antioxidant and other activities.[9] Diallyl sulphide (DAS) and Diallyl

disulphide (DADS) stimulate the activity of glutathione peroxidase (GPX), which increases the activity of superoxide dismutase (SOD), an enzyme involved in the fight against free radicals.[28] Thus, their radical scavenging properties implicate flavonoids in preventing oxidative damage to cellular molecules caused by ROS during the development of BPH. In addition, AS fresh juice extract administered as a preventive measure decreased MDA activity and significantly increased SOD expression. This may be due to the early administration of garlic, which, through its flavonoids, increases the early intracellular activity of superoxide dismutase (SOD), eliminating or neutralising ROS. The antioxidant effects of SA juice are supported by the chemical composition of the extract, which is rich in flavonoids, notably Diallyl disulphide (DADS), Diallyl trisulphide (DATS) and S-allylcysteine (SAC), which inhibit the polyamines needed for cell division, increase the breakdown of testosterone, which is necessary for the growth of prostate cancer, and reduce the level of specific antigen (PSA).[29]

CONCLUSION

The present study showed that fresh AS juice has both preventive and curative properties on TE-induced BPH in rats. These effects are reflected in a decrease in prostate index, testosterone and PSA levels, a decrease in MDA activity and an increase in SOD levels. The biological activity of fresh AS juice is thought to be attributable to the presence of certain chemical compounds such as flavonoids and polyphenols. Further studies could clarify the mechanism of this antiproliferative activity of AS and support the development of new anti-cancer therapeutic strategies based on this plant.

Authors' contributions

All the authors contributed to this work. The authors of this article have read and approved its content.

Conflicts of interest

The authors declare that there are no conflicts of interest in relation to this work.

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