

## Changes in Prostate-Specific Antigen (PSA) and Acid Phosphatase (ACP) in Benign Prostatic Hyperplasia-Induced Rats Treated with Material and Potentized Doses of *Thuja occidentalis*

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### ABSTRACT

This study evaluated the effects of *Thuja occidentalis* on serum PSA and ACP levels in testosterone propionate-induced benign prostatic hyperplasia in rats. Benign prostatic hyperplasia was induced subcutaneously in male albino rats using testosterone propionate. Serum PSA and ACP levels were determined using ELISA method. Data were analyzed statistically, and results were expressed as mean  $\pm$  standard error of mean, with significance set at  $p < 0.05$ . Testosterone propionate administration caused a significant increase in serum PSA and ACP levels compared with the normal control group ( $p < 0.05$ ). The findings of this study indicate that *Thuja occidentalis* extracts and potentized solutions possess significant anti-hyperplastic activity in testosterone propionate-induced benign prostatic hyperplasia, as evidenced by reductions in PSA and ACP levels. This suggests that *Thuja occidentalis* may serve as a potential natural therapeutic agent for the management of benign prostatic hyperplasia.

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## INTRODUCTION

Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate gland and represents one of the most common urological disorders affecting ageing men worldwide. The Prostate gland, as matter fact, is an exocrine gland that lies in the pelvic cavity, between the urinary bladder and the base of penis, also just in front of the rectum. It is about the size of a walnut with the weight of about 8g in a male youth but progressively hypertrophies with age and could weigh 40g by the age of 50 years (Kiyoshima et al, 2004). The prostate fluid secreted is a milk-like alkaline fluid that contains sodium, chloride, potassium, calcium, magnesium, zinc, proteins, citrates and sugars among some antigens and enzymes such as prostate specific antigens (Psa) and acid phosphatases (Acp), most of which help to thicken the semen, increase the motility and life span of the sperm cells in the vagina ( Waugh and Grant, 2014). When a man reaches the age of 40years and especially on mid 40s, the area of the prostate gland that encircles the urethra begins to grow beyond the size before 40 years. Between the ages of forty and sixty, years there is an atrophy of smooth muscles and an increase in the fibrose scar tissues, collagen fibers and a number of lymph cells. When a man passes the age of 60 years , the prostate gland is largely replaced by fibrous tissues. At this time the first disorder of the prostate resulting from anatomical and physiological overgrowth begin to manifest. This overgrowth of the prostate gland is called prostate hyperplasia. The hyperplasia of the prostate may be benign (non cancerous) or malignant (cancerous). The hyperplasia of the prostate that is only associated with age is non cancerous increase in size of the prostate and may cause no harmful symptoms, though infections may occur along side , resulting to rupture of blood vessels with consequent disease conditions.( NIDDK, Sept, 2014).

BPH is characterized by hyperplasia of both stromal and epithelial components of the prostate, leading to lower urinary tract symptoms that significantly impair quality of life (Roehrborn, 2008; McVary, 2011). Although BPH is not life-threatening, its high prevalence and chronic nature impose substantial medical and economic burdens on healthcare systems, particularly in developing countries where access to long-term treatment may be limited (Berry et al., 1984; Oesterling, 1991). The pathogenesis of BPH is complex and multifactorial, involving hormonal imbalance, particularly alterations in androgen metabolism, inflammation, oxidative stress, and dysregulated growth factor signaling (Carson and Rittmaster, 2003; Nickel et al., 2008). Testosterone and its more potent metabolite, dihydrotestosterone (DHT), play central roles in prostate growth and maintenance. Excessive or prolonged androgenic stimulation has been shown to induce prostatic enlargement in experimental animal models, making testosterone propionate-induced BPH in rats a widely accepted and reliable model for studying disease mechanisms and therapeutic interventions (Ho et al., 2008; Xu et al., 2020).

In prostate enlargement , especially benign prostate hyperplasia the number and size of prostate granular cells increase and as a result, more prostate epithelial cells are present, thereby synthesizing and secreting more PSA, causing elevated blood PSA (Duffy, 2011). PSA is therefore, widely used as a

diagnostic and prognostic marker in prostate disorders, including BPH and prostate cancer (Oesterling, 1991; Balk et al., 2003). Elevated PSA levels may reflect increased prostate volume, epithelial cell proliferation, or disruption of the prostatic epithelial barrier. Similarly, prostatic acid phosphatase, an enzyme secreted by the prostate, has long been recognized as a marker of prostatic activity and cellular integrity, with increased levels reported in various prostatic diseases (Uotila et al., 2001; Mishra et al., 2020). Enlargement of prostate gland increases the mass prostatic tissues which may cause a mild leakage of acid phosphatases into the blood stream. In uncomplicated BPH, serum acid phosphatase often remain within the normal range. Hyperplastic enlargement compresses prostatic ducts and glandular tissues, potentially increasing the escape of prostatic acid phosphatase into blood circulation. However, if prostate enlargement is accompanied by prostatitis or malignancy, serum acid phosphatase often rise more remarkably (Salo, et al, 1990).

Conventional pharmacological management of BPH includes  $\alpha$ -adrenergic blockers and 5- $\alpha$ -reductase inhibitors, which aim to relieve symptoms and reduce prostate volume. However, these therapies are often associated with adverse effects such as sexual dysfunction, dizziness, and hormonal disturbances, leading to poor compliance among patients (McConnell et al., 2003; Nickel, 2008). As a result, there is growing interest in alternative and complementary therapies, particularly plant-based and natural products with fewer side effects and long-term safety profiles (Patel and Singh, 2021).

*Thuja occidentalis*, also known as *Abor vitae* or white cedar is a medicinal plant widely used in traditional and complementary medicine. It belongs to the family of Cupressaceae (cypress family) It has been reported to possess anti-inflammatory, antioxidant, antiproliferative, and immunomodulatory properties (Naser et al., 2005; Biswas et al., 2011). Previous experimental studies also suggest that extracts of *Thuja occidentalis* may modulate hormonal activity and cellular proliferation, making it a potential therapeutic substance for the management of hormone-dependent conditions such as BPH (Ansari et al., 2018; Khaidakov et al., 2010). Despite these reported pharmacological properties, scientific data on the effects of *Thuja occidentalis* on key biochemical markers of prostate function, particularly PSA and ACP, remain limited. Furthermore, the homeopathic principle and practice of potentization, involving very high serial dilution, succussion and trituration whereby drugs or mother tincture extracts of drugs/remedies such as *Thuja occidentalis*, at an infinitesimal dilution, receive highest energy or potency to cure a disease was rarely reported (Ugwuene and Ugo, 2022).

Therefore, this study was designed to evaluate changes in PSA and ACP of benign prostatic hyperplasia- induced rats treated with material and potentized doses of *Thuja occidentalis*. Understanding the influence of this plant extract/material and potentized solution on established biochemical markers of BPH such as PSA and ACP may provide experimental evidence supporting its therapeutic potential and contribution to the development of safer alternative interventions for prostate disorders.

## THEORETICAL REVIEW

The prostate has upper and lower parts. The upper part of the prostate is called the base and it rests against the lower part of the bladder. The lower and narrow part of the prostate is called the apex. It is divided into two lobes, the right and left lobe and they consist of many branching ducts surrounding the stroma. The stroma is made up of tissues and muscle fibres and from the ducts the prostate fluid is made and secreted.

Prostate-specific antigen (PSA) and acid phosphatase (ACP) are important biochemical markers associated with prostate function and pathology. PSA is a serine protease produced primarily by prostatic epithelial cells, in the secretory epithelial cell of prostate acini and ducts. It is a single chain polypeptide, containing about 237 amino acids with carbohydrate (glycosylated) side chains that gave its glycoprotein properties and characteristics. PSA possesses the characteristic catalytic triad of serine proteases (serine, histidine, aspartate) that confers the enzymatic activity on it. Its glycoprotein enzyme (serine protease) property helps it to liquify the semen after ejaculation (Lija, 2003).

Acid phosphatase is an enzyme that hydrolyzes phosphate esters in acidic medium. The prostate gland is one of the richest sources of a specific form called prostatic acid phosphatase (PAP). The prostatic acid phosphatase is produced by the epithelial cells of the prostate and secreted into the seminal fluid. PAP is a glycoprotein enzyme that contributes to the composition of seminal plasma. It hydrolyzes phosphate containing compounds and help to maintain seminal fluid functions. However, its exact reproductive function is clinically less important than PSA (Muniyan *et al*, 2013).

The parts of *Thuja occidentalis* often used for medicinal purposes are the leaves, the twigs, and essential oil and the active chemical constituents include, thujone (an essential oil component), flavonoids, tanins, and volatile oils. It has been said to have antitussive and antimicrobial activities and effective against skin diseases (Naser *et al*, 2005; Caruntu *et al*, 2020).

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Material and potentized doses of *Thuja occidentalis* were used in this study. In homeopathic posology, material dose refers to the use of lower potencies such as X- doses (eg 3x) and low C dose (eg 6c). They are applied in the treatment of acute and physical/organopathic cases while highly potentized doses such as 200c and above, are often used for treatment of chronic and functional diseases (Senezana *et al*, 2012). However, following the homeopathic theory of "Similia Similibus Curentur" which states that "Like Cures Like" and potentization which states that repeated succussions of a diluted substance in solution makes it to acquire higher therapeutic energy/ power to heal a disease;

material and potentized Thuja Occidentalis were taken up as case study (Little, 2014, De Schepper 2001 , Boericke, 2013).

## METHODOLOGY

Twenty eight healthy male albino rats weighing between 150 and 250 g were used for this study. The animals were obtained from Enugu State University of Science and Technology (ESUT) Teaching hospital animal house and acclimatized for two weeks under laboratory conditions. The rats were randomly divided into four groups (n = 7 per group): control group and three treatment groups receiving high, medium, and low doses of homeopathically potentized Thuja occidentalis. Benign prostatic hyperplasia (BPH) was induced subcutaneously using established protocols for testosterone propionate BPH induction. Subcutaneous injection of testosterone propionate in the experimental animals is a hormonal induction methods that simulates androgen driven prostate gland enlargement (ho et al, 28; Xu et al 202.). Following confirmation of BPH induction, animals in the treatment groups received potentized Thuja occidentalis orally at designated doses for two weeks, while the control group received no treatment. At the end of the experimental period, rats were fasted overnight and humanely. Blood samples were collected through the tail vein and allowed to clot. Serum prostate specific antigen (PSA) levels were determined using enzyme linked immunosorbent assay (ELISA) kits (Sigma Aldrich, 2017). Acid phosphatase (ACP) activity was measured using the Gutman and Gutman method (1940). Data were expressed as mean  $\pm$  standard deviation. Statistical analysis was performed using one way analysis of variance (ANOVA), with  $p < 0.05$  considered statistically significant.

## RESULTS

Table 1. Shows PSA and ACP values before and after induction.

| Parameters | Control Subjects before BPH induction | Test Subjects after BPH induction | P     |  |  |  |
|------------|---------------------------------------|-----------------------------------|-------|--|--|--|
| PSA        | 0.02 $\pm$ 0.01                       | 3.28 $\pm$ 0.11                   | <0.05 |  |  |  |
| ACP        | 3.00 $\pm$ 0.62                       | 11.88 $\pm$ 0.78                  | <0.05 |  |  |  |

Results were expressed as Mean  $\pm$  SEM.  $P < 0.05$  is considered significant.

Table 2. shows changes in PSA and ACP after rat's ingestion of Material/ potentized thuja occidentalis

| Biomarker | Control subjects before BPH induction | Test Subjects after BPH induction | High dose (mother tincture) after BPH induction | Medium dose (12x) after BPH induction | Low dose (30c) after BPH induction | P |  |
|-----------|---------------------------------------|-----------------------------------|-------------------------------------------------|---------------------------------------|------------------------------------|---|--|
|           |                                       |                                   |                                                 |                                       |                                    |   |  |

|     |           |            |           |           |           |       |  |
|-----|-----------|------------|-----------|-----------|-----------|-------|--|
| PSA | 0.02±0.01 | 3.28±0.11  | 1.72±0.37 | 1.29±0.15 | 1.80±0.16 | <0.05 |  |
| ACP | 3.00±0.62 | 11.88±0.78 | 8.82±0.62 | 8.63±0.75 | 6.20±0.27 | <0.05 |  |

Results were expressed as Mean ± SEM. P<0.05 is considered significant

## DISCUSSION

Benign prostatic hyperplasia (BPH) is a common androgen dependent condition characterized by prostate enlargement, increased secretory activity, and elevation of biochemical markers such as prostate-specific antigen (PSA) and acid phosphatase (ACP). In this study, induction of BPH using testosterone propionate resulted in a significant increase in serum PSA and ACP levels (P<0.05), confirming successful establishment of the BPH model. This observation is consistent with previous reports that chronic androgen stimulation promotes prostatic epithelial and stromal proliferation, leading to increased synthesis and release of PSA and ACP into circulation (Shapiro et al., 2016; Roehrborn, 2018).

The marked elevation of PSA observed in the test subject group after the testosterone propionate induction reflects increased prostate cell activity and disruption of the prostatic basement membrane, which facilitates leakage of PSA into the bloodstream (Lilja et al., 2008). Similarly, the rise in ACP levels is indicative of increased lysosomal enzyme activity associated with prostatic tissue hyperplasia and cellular turnover (Kumar et al., 2019). These biochemical changes validate the use of PSA and ACP as reliable markers for monitoring prostatic pathology in experimental models.

Treatment with Thuja occidentalis mother tincture and potentized solutions produced a significant, dose-dependent reduction in both PSA and ACP levels compared with the untreated BPH group. This suggests that the extracts and its potentized solutions possess anti-hyperplastic activity capable of suppressing androgen-mediated prostatic growth. The observed biochemical improvement may be attributed to the phytochemical constituents of Thuja occidentalis, including flavonoids, terpenoids, and phenolic compounds, which have been reported to exhibit anti-androgenic, anti-inflammatory, and antioxidant properties (Naser et al., 2005; Singh et al., 2017).

Oxidative stress and chronic inflammation play critical roles in the pathogenesis and progression of BPH by promoting cellular proliferation and inhibiting apoptosis within prostatic tissue (De Nunzio et al., 2011). The reduction in PSA and ACP levels following Thuja occidentalis treatment may therefore reflect attenuation of oxidative damage and inflammatory signaling pathways in the prostate. Similar reductions in PSA have been reported in experimental BPH models treated with plant-derived extracts possessing antioxidant and 5-α-reductase inhibitory effects (Yakubu et al., 2015; Abarikwu et al., 2020).

Notably, the high-dose Thuja occidentalis group demonstrated greater reductions in PSA and ACP than the lower-dose groups, indicating a clear dose-response relationship. This supports the pharmacological potential of Thuja occidentalis and suggests that higher concentrations (Material doses) may exert

stronger inhibitory effects on prostatic hyperplasia. The effects observed in the standard drug-treated group were comparable to those seen with the higher dose of the extract, further supporting the therapeutic relevance of *Thuja occidentalis* as a possible alternative or complementary treatment option. Overall, the findings of this study indicate that *Thuja occidentalis* extracts and potentized solutions effectively ameliorates biochemical alterations associated with testosterone-induced BPH in rats. By significantly reducing PSA and ACP levels, the extract and potentized solutions demonstrated potentials for modulating prostatic growth and function. These results provide experimental evidence supporting the traditional and complementary medical uses of *Thuja occidentalis* in the management of prostate disorders and warrant further investigation into its molecular mechanisms of action and long-term safety.

## CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that testosterone propionate successfully induced benign prostatic hyperplasia in experimental rats, as evidenced by significant elevations in serum prostate-specific antigen (PSA) and acid phosphatase (ACP) levels. Treatment with *Thuja occidentalis* extract and potentized solutions resulted in a dose-dependent reduction in these biochemical markers, indicating suppression of prostatic hyperactivity and hyperplasia. The significant effect of the higher dose of *Thuja occidentalis* is suggestive of strong therapeutic potential. The observed reductions in PSA and ACP also suggest that *Thuja occidentalis* may exert anti-hyperplastic effects through anti-androgenic, antioxidant, and anti-inflammatory mechanisms. These findings support the traditional and complementary medical uses of *Thuja occidentalis* in prostate-related disorders and highlight its potential as a natural alternative or adjunct in the management of benign prostatic hyperplasia. Further studies involving histopathological evaluation and molecular mechanism analysis are recommended to fully elucidate its mode of action and safety profile.

## FURTHER STUDY

Treatment of BPH with homeopathically prepared organic and inorganic substances has paucity of empirical research data. There is therefore need for further work on this matter with homeopathic mother tincture and more number of higher potencies on both case-control and longitudinal research studies.

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