

# Relative Influence of *Ageratum conyzoides* L. Plant Extracts on Testosterone Induced Benign Prostatic Hyperplasia in Mice

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## ABSTRACT

**Purpose:** The present study was designed to investigate the inhibitory efficacy of aqueous and ethanolic extracts of *Ageratum conyzoides* L. (AC, Family asteraceae) on testosterone induced BPH in male mice. **Materials and Methods:** Mice were randomly divided into five groups ( $n=6$ ): negative control group received corn oil, BPH model group receive testosterone (5mg/kg/day, S.C) dissolved in corn oil. Another group received standard BPH drug finasteride (5 mg/Kg/day, I.P) along with testosterone injection (5mg/kg/day, S.C). The other two group of animals received either aqueous or ethanolic AC plant extracts (100, 500, 1000 and 2000 mg/kg, I.P) along with testosterone (5mg/kg/day, S.C) consecutively for 28 days. All animals were sacrificed at the end of the study period and prostate weight and prostatic index were determined, followed by histopathological examination of the prostate gland. **Results:** The result of the present study indicated that treatment with either plant extracts caused significant reduction in prostate weight and prostatic index when compared to only testosterone treated animal group. The percentage of inhibition of prostate growth was found to be correlated in a dose dependent manner in plant extract treated groups. Histopathological examination of prostate gland revealed AC plant extracts treatment significantly reduced the glandular hyperplasia, epithelial thickness and stromal hyperplasia in the prostate gland of the treated animals. No significant elevation in serum ALT and creatinine level was recorded in any groups during the study period. **Conclusion:** This study hence indicate the therapeutic potential in *Ageratum conyzoides* L. plant extract in testosterone induced BPH model in mice.

**Keywords:** Benign prostatic hyperplasia, Prostate, Inhibition, Testosterone, *Ageratum conyzoides*, Plant extracts.

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## INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a non-malignant progressive, androgen dependent disease resulting in enlargement of the prostate gland, which affects 50% of the male once they reached their fifties and more than 80% of male over the age of eighties.<sup>[1,2]</sup> It is associated with proliferation of epithelial and stromal cell that occurs in the transition zone of the prostate gland.<sup>[3]</sup> Clinically BPH is identified by bladder outflow obstruction and urinary tract symptoms (LUTs) that includes urgency to urinate, frequent urination, sensation of incomplete bladder emptying and nocturia that lead to an increased risk of obstruction of the urethra, urinary retention and urinary tract

infection.<sup>[4,5]</sup> The mechanism underlying the pathogenesis of BPH is not clearly understood but aging and androgen specially dihydrotestosterone (DHT), which is known to play a significant role in the diseases progression.<sup>[6]</sup>

*Ageratum conyzoides* L. known as Billy goat weed belongs to the family Asteraceae, is an invasive plant which is commonly used in many parts of the world for its medicinal, pharmacological and therapeutic properties. It acts as anti-inflammatory, analgesic and anti-pyretic, antioxidant, antibacterial, anti-poliferative, anti-tumor, anti-cancer.<sup>[7-12]</sup> Given these effect, we consider that *Ageratum conyzoides* L. could be an effective drug for the treatment of BPH. Many previous studies have investigated the pharmacological effect of *Ageratum conyzoides* L., however few study has reported the possible protective effect against testosterone induced BPH in mice model. Therefore, the present study was designed to investigate the efficacy of aqueous and ethanolic plant extracts of *Ageratum conyzoides* L. in testosterone induced benign prostate hyperplasia in mice model.



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## MATERIALS AND METHODS

### Chemicals and Reagents

Testosterone propionate (TP) was purchased from Sigma-Aldrich, and Finasteride was purchased from Dr. Reddy's Laboratory, India. All other chemicals used in the experiment were of analytical grade.

### Plant materials and preparation of plant extract:

The fresh leaves of *Ageratum conyzoides* L. were collected from Rajiv Gandhi University campus, Itanagar and were identified and authenticated by Scientist Dr. Umeshkumar L. Tiwarifrom Botanical Survey of India, Arunachal Pradesh. A voucher specimen (TJ02/BSI-APRC/2018/29851-ARUN) was deposited in ARUN BSI, Itanagar. The leaves were washed, shades dried and blended into fine powder. The powdered leaves (10g in 50 ml) were extracted with distilled water and ethanol for 60 min at room temperature using rotary evaporator and evaporated to dryness. The dried extract collected was further diluted with doubledistilled water and were filtered with Whatmann filter paper (No.42) and kept in freezer until used.

### Animals

Adult male Swiss albino mice, weighing 30±5g (Animal house facility, Department of Zoology, Rajiv Gandhi University, Itanagar were housed in polypropylene cage bedding with paddy husk under at a standard environmental condition of constant temperature (24±2°C), humidity (65-70%) and a 12:12hr light/dark cycle. All animals were offered a standard laboratory diet and water *ad libitum*. Animals were allowed to acclimatize for a week prior to experiment. All experimental procedure was performed in accordance with the Institutional animal ethical committee guideline for the care and use of laboratory animal (IEAC/RGU/19/18).

### Induction of BPH in mice model

BPH was induced in randomly selected mice (Positive Control group,  $n=18$ ) by subcutaneous (S.C) injection of testosterone propionate (5 mg/kg/day) dissolved in corn oil for 21 days.<sup>[13-15]</sup> The negative control group ( $n=18$ ) received same volume of S.C injection of corn oil for 21 days. Six number ( $n=6$ ) of animals from both BPH or positive control group and negative control were sacrificed after 7, 14 and 21 days of treatment by cervical dislocation under ketamine hydrochloride anesthesia. Prostate, Liver and Kidney from each animal was excised, immediately washed and rinsed with ice cold normal saline. Ventral lobe of the prostate was fixed in 10% neutral buffered formalin for histopathological studies.

### Dose response study of AC plant extract in testosterone induced BPH model in mice

To determine the effect of AC plant extracts on testosterone induced BPH, randomly selected mice ( $n=60$ ) were first treated with testosterone (5 mg/Kg/day, S.C) for 2 weeks to induce BPH.<sup>[14]</sup> Another group of mice ( $n=6$ ) that act as negative control was treated only with corn oil for the same duration. The testosterone treated group of animals was further subdivided and treated with either aqueous or ethanolic plant extracts of *Ageratum conyzoides* L. (100, 500, 1000 and 2000 mg/kg/day of B.W) for another 2 weeks. A group of testosterone treated mice ( $n=6$ ) served as BPH model group or positive control group without any treatment and another group of testosterone treated mice ( $n=6$ ) received standard BPH drug Finasteride (5 mg/kg/day, I.P). After completion of treatment, the animals were sacrificed under ketamine hydrochloride anesthesia and blood samples was collected from the right atrium of the heart, allowed to clot (20 - 30 min at R.T) and then the serum was separated (1000 rpm, 15 min) for the estimation of total ALT and Creatinine. Prostate, Liver and Kidney were immediately removed, weighted and were fixed in 10% neutral buffer formalin for histological studies.

### Determination of Prostatic index and percentage of inhibition

Prostatic index which gives an idea of relative prostate weight in an animal and percentage of inhibition, exhibited by different plant extracts are calculated as follows.<sup>[16,17]</sup>

$$\text{Prostatic Index (PI)} = \frac{\text{Total Prostate weight (g)}}{\text{Final body weight (g)}} \times 100$$

$$\text{Percentage of Inhibition (\%)} = 100 - \left[ \frac{T - NC}{PC - NC} \right] \times 100$$

Where, NC: Negative Control, PC: Positive Control and T: Treated Group

### Estimation of serum Alanine aminotransferase (ALT) and Creatinine level

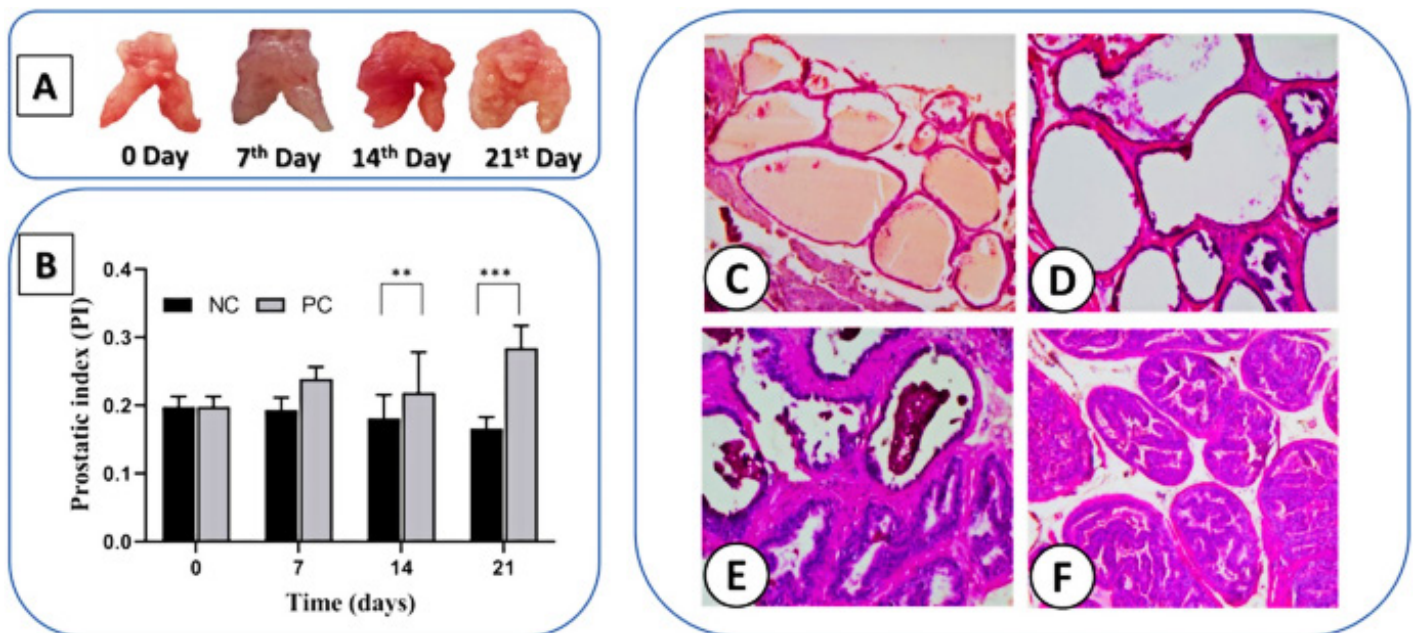
Serum Alanine aminotransferase (sALT) and Creatinine level, which is used as a potential biomarker for hepatic injury and detect renal dysfunction respectively, was estimated using a commercial kit (Coral Clinical system, Goa, India) based on Reitman and Frankel (1957) and alkaline picrate based on Jaffe reaction method.

### Histological analysis

The ventral lobe of the prostate were fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded alcohol, embedding in paraffin wax, cut into 5µm thick section and stained with hematoxyline and eosin.<sup>[18]</sup> The section were mounted with DPX

**Table 1: Induction of BPH model in mice by subcutaneous injection of testosterone (5mg/kg). Values are expressed as Mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Newman Keuls multiple comparison test. #p <0.05 when compared with negative control.**

Groups	Initial body weight (g)	Final body weight (g)	Diff. in body weight (g)	Prostate weight (g)	Relative liver weight (g)	Relative kidney weight (g)
Negative Control	35.28 ± 0.34	35.28 ± 0.34	NA	0.070 ± 0.005	4.126 ± 0.405	1.379 ± 0.093
07 Days						
Negative Control	32.28 ± 0.58	32.92 ± 1.20	0.64 ± 0.90	0.063 ± 0.003	4.873 ± 0.281	0.929 ± 0.062
Positive Control (T <sub>5mg/kg</sub> )	35.76 ± 2.30	38.63 ± 3.81	2.86 ± 1.71	0.093 ± 0.014	4.964 ± 0.102	0.877 ± 0.033
14 Days						
Negative Control	33.97 ± 1.35	34.75 ± 1.58	0.78 ± 0.61	0.063 ± 0.013	4.884 ± 0.216	1.106 ± 0.183
Positive Control (T <sub>5mg/kg</sub> )	34.83 ± 2.56	34.73 ± 3.40	-0.09 ± 0.96	0.073 ± 0.014	5.102 ± 0.278	1.007 ± 0.103
21 Days						
Negative Control	34.04 ± 1.29	34.07 ± 1.27	0.02 ± 0.02	0.056 ± 0.006	4.617 ± 0.352	1.635 ± 0.119
Positive Control (T <sub>5mg/kg</sub> )	38.08 ± 3.34	40.29 ± 2.13	2.21 ± 1.41	0.113 ± 0.008 <sup>#</sup>	4.836 ± 0.209	0.906 ± 0.089



**Figure 1:** Induction of BPH in mice model by subcutaneous injection of testosterone (5mg/kg/day) and subsequent effect on (A) prostate weight and (B) prostatic index. Values are expressed as Mean ± SEM (n=6). The data were analyzed by one-way ANOVA followed by Newman Keuls multiple comparison test,  $p < 0.05$  considered as significant. (C-F) Histopathological changes in the prostate tissues of testosterone (5mg/kg/day, S.C) induced BPH model in mice at different time point of interval (0, 7, 14 and 21 days). H&E stain, X400 magnification.

and coverslip, examined and photographed under Leica DM2000 LED microscope for recording any histological changes.

### Statistical analysis

Data were expressed as mean ± S.E.M. Statistical analysis was done by using one way analysis of variance (ANOVA) followed by Newman-Keul multiple comparison tests using Graph pad prism statistical software version 8,  $p < 0.05$  considered as statistically significant.

## RESULTS

### Effect of Testosterone Treatment in Mice

Administration of testosterone significantly increases prostate weight ( $0.113 \pm 0.008$ ,  $^*p < 0.05$ ) and prostatic index ( $^{**}p < 0.01$ ,  $^{***}p < 0.001$ ) in mice in a time dependent manner when compared to negative control group as presented in Table 1 and Figure 1A and 1B. No significant difference in body weight, relative liver and kidney weight was observed after testosterone treatment.

**Table 2: Dose response study of AC<sub>Aqueous</sub> and AC<sub>EthOH</sub> plant extracts of *Ageratum conyzoides* L.**

Groups	Initial body weight (g)	Final body weight (g)	Diff. in body weight (g)	Prostate weight (g)	Prostatic Index	% of inhibition
Negative Control	33.71 ± 0.97	33.54 ± 1.04	-0.16 ± 0.19	0.05 ± 0.008	0.147 ± 0.021	
BPH/Positive Control (T <sub>5mg/kg</sub> )	31.92 ± 1.38	34.42 ± 1.17	2.49 ± 1.70	0.097 ± 0.004 <sup>###</sup>	0.283 ± 0.014 <sup>###</sup>	
T <sub>5mg/kg</sub> + Finasteride (3mg/kg)	30.27 ± 0.11	29.74 ± 0.58	-0.52 ± 0.53	0.052 ± 0.006 <sup>###</sup>	0.177 ± 0.024 <sup>**</sup>	77.38 ± 18.33
<b>AC<sub>Aqueous</sub></b>						
T <sub>5mg/kg</sub> + AC <sub>Aq</sub> (100mg/kg)	34.51 ± 2.50	34.11 ± 2.19	-0.40 ± 0.39	0.07 ± 0.004 <sup>**</sup>	0.205 ± 0.007 <sup>**</sup>	56.98 ± 5.61
T + AC <sub>Aq</sub> (500mg/kg)	34.43 ± 0.92	35.27 ± 1.20	0.84 ± 1.15	0.072 ± 0.002 <sup>**</sup>	0.205 ± 0.002 <sup>*</sup>	57.35 ± 2.01
T + AC <sub>Aq</sub> (1000mg/kg)	29.60 ± 1.81	32.18 ± 1.60	2.57 ± 1.05	0.055 ± 0.006 <sup>###</sup>	0.170 ± 0.017 <sup>**</sup>	82.90 ± 12.51
T + AC <sub>Aq</sub> (2000mg/kg)	30.51 ± 0.81	33.15 ± 1.28	2.63 ± 1.03	0.052 ± 0.009 <sup>###</sup>	0.156 ± 0.022 <sup>###</sup>	93.56 ± 16.38
<b>AC<sub>EthOH</sub></b>						
T + AC <sub>EthOH</sub> (100mg/kg)	32.03 ± 0.86	33.39 ± 0.86	1.35 ± 0.79	0.065 ± 0.006 <sup>**</sup>	0.196 ± 0.024 <sup>**</sup>	63.97 ± 18.03
T + AC <sub>EthOH</sub> (500mg/kg)	33.41 ± 1.46	34.85 ± 2.08	1.44 ± 1.15	0.065 ± 0.002 <sup>**</sup>	0.187 ± 0.007 <sup>**</sup>	70.95 ± 5.32
T + AC <sub>EthOH</sub> (1000mg/kg)	30.68 ± 0.97	31.49 ± 1.11	0.80 ± 1.11	0.05 ± 0.004 <sup>###</sup>	0.159 ± 0.015 <sup>###</sup>	90.99 ± 11.42
T + AC <sub>EthOH</sub> (2000mg/kg)	29.58 ± 0.46	33.25 ± 0.90	3.67 ± 1.36	0.042 ± 0.002 <sup>###</sup>	0.127 ± 0.006 <sup>###</sup>	114.52 ± 4.70

On body weight, prostate weight and prostatic index in testosterone (5mg/kg/day, S.C) induced BPH model in mice. Values are expressed as Mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Newman Keuls multiple comparison test. <sup>###</sup> p<0.001 when compared with negative control, <sup>\*</sup> p<0.05, <sup>\*\*</sup> p<0.01 and <sup>###</sup> p<0.001 when compared with BPH model.

Histopathological examination of the prostate tissue of negative control group showed, the organ is composed of regular tightly packed acini lined by single cuboidal epithelium (Figure 1 C). But in positive control group or BPH model group testosterone does caused significant alteration in the histological organization of the prostate tissues as depicted in Figure 1 (D-F). The proliferation of stromal tissues has been quite evident after 7 days of testosterone treatment (Figure 1 D) and severity of proliferation increases with an increase in the number of days of testosterone treatments (Figure 1 E and F). After 21 days of testosterone treatment, due to continuous proliferation of epithelial lining of the prostate acini, it gives the appearance of intraepithelial neoplasia projected into the lumen (Figure 1 D). Due to the inward growth of the epithelial hyperplasia, the volume of glandular lumen ultimately decreases in BPH model group when compared to the negative control group (Figure 1 D).

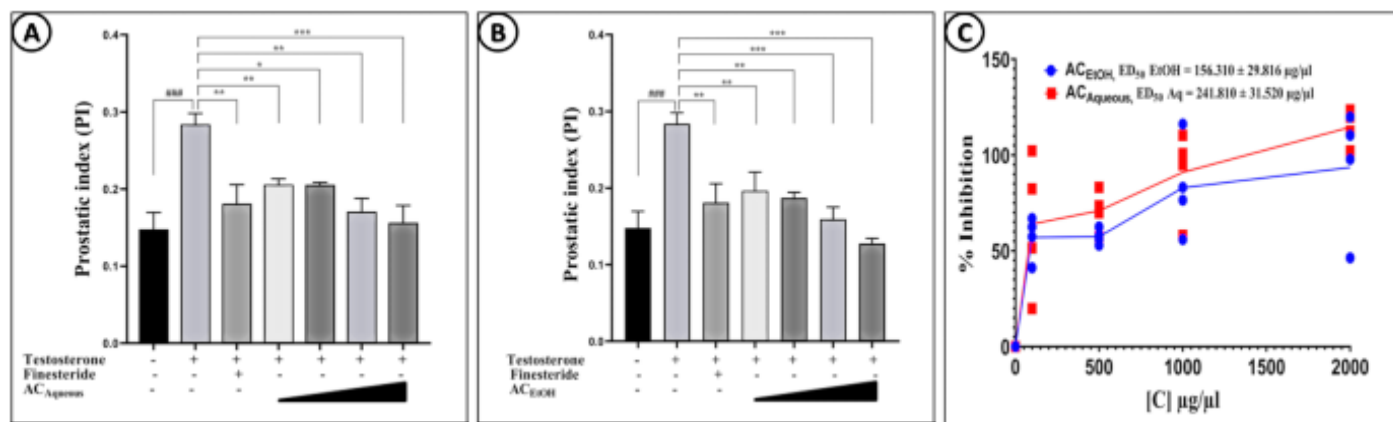
### Effect of AC plant extract treatment on testosterone induced BPH in mice

The prostate enlargement is commonly used indicator for BPH development (Table 2). In a dose response study, animals treated with testosterone (5mg/kg/day, S.C) showed a significant increase in prostate weight (0.097 ± 0.004, p<0.001) when compared with the negative control (0.05 ± 0.008). However the effect of testosterone was inhibited in animals treated with finasteride (5 mg/kg/day, I.P, 0.052 ± 0.006, p<0.001). Administration of

AC<sub>aqueous</sub> plant extract also exhibited significant dose dependent reduction in prostate weight (0.07 ± 0.004; 0.072 ± 0.002, p<0.01; 0.055 ± 0.006; 0.052 ± 0.009, p<0.001) when compared with the testosterone treated BPH mice model. Similarly, the mice treated with AC<sub>EthOH</sub> plant extract exhibited significant dose dependent reduction in prostate weight (0.065 ± 0.006; 0.065 ± 0.002, p<0.01; 0.05 ± 0.004; 0.042 ± 0.002, p<0.001) when compared with the testosterone treated group (Table 2).

The prostatic index was also significantly decreased in both plant extracts treated group and finasteride group when compared with the BPH positive group (Figure 2A and 2B). It was observed that both *Ageratum conyzoides* L. plant extract decreases the mean prostate weight and prostatic index in dose dependent manner (Table 2 and Figure 2A and 2B). Finasteride treatment caused percentage inhibition of 77.38% in prostate index while in both plant extracts treated group the exhibited a dose dependent increase of percentage inhibition in the prostate index. The percentage inhibition was found to be in the range of 56.98%, 57.35%, 82.90% and 93.56% when treated with AC<sub>aqueous</sub> plant extract (Figure 2C). Similarly AC<sub>EthOH</sub> plant extract treatment caused percentage inhibition of 63.97%, 70.95%, 90.99% and 114.52% respectively (Figure 2C).

From the dose response study the effective dose (ED<sub>50</sub>) of aqueous and ethanolic plant extract of *Ageratum conyzoides* L. in testosterone induced benign prostate hyperplasia in mice model was calculated as 241.81µg/µl and 156.31µg/µl (Figure



**Figure 2:** (A) Prostatic index in the AC<sub>aqueous</sub> and (B) AC<sub>EtOH</sub> treated groups. (C) Effective dose (ED<sub>50</sub>) of both plant extract calculated from percentage of inhibition using graph padprism 8 software. Values are expressed as Mean ± SEM (n=6). ###p<0.001 when compared with negative control; \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 when compared with testosterone treatment.

2C). No significant change in body weights was found in different experimental groups.

Histopathological changes in the prostate tissues of different experimental group of animals are presented in Figure 3 (A-K). No stromal hyperplasia was observed in negative control group and the lumen of prostate acini were normal surrounded by regular sized cuboidal epithelial cells (Figure 3A). In the BPH model group, significant stromal and glandular hyperplasia was recorded with proliferation of acinar epithelial cells, thickening of the glandular epithelium and decrease in luminal space as compared to the negative control group (Figure 3B). Epithelial hyperplasia was significantly reduced and improvement in luminal space was observed in the finasteride treated group (Figure 3C). Treatment with both plant extracts (AC<sub>aqueous</sub> and AC<sub>EtOH</sub>) caused significant dose dependent reduction in epithelial hyperplasia in the prostate acini as well as stromal hyperplasia when compared with the BPH group (Figure 3D-K).

No significant difference in testicular index, relative liver weight and relative kidney weight was observed. However, in group treated with AC<sub>aqueous</sub> (2000 mg/kg/day) increase in relative liver weight (6.680 ± 0.086, p<0.001) and finasteride treated group (5 mg/kg/day) increase in relative kidney weight (1.929 ± 0.065, p<0.05) was recorded when compared with testosterone induced BPH model group (Table 3). However serum ALT level and creatinine level which is a marker enzyme for liver toxicity and kidney damage did not differ significantly among the experimental groups (Table 3).

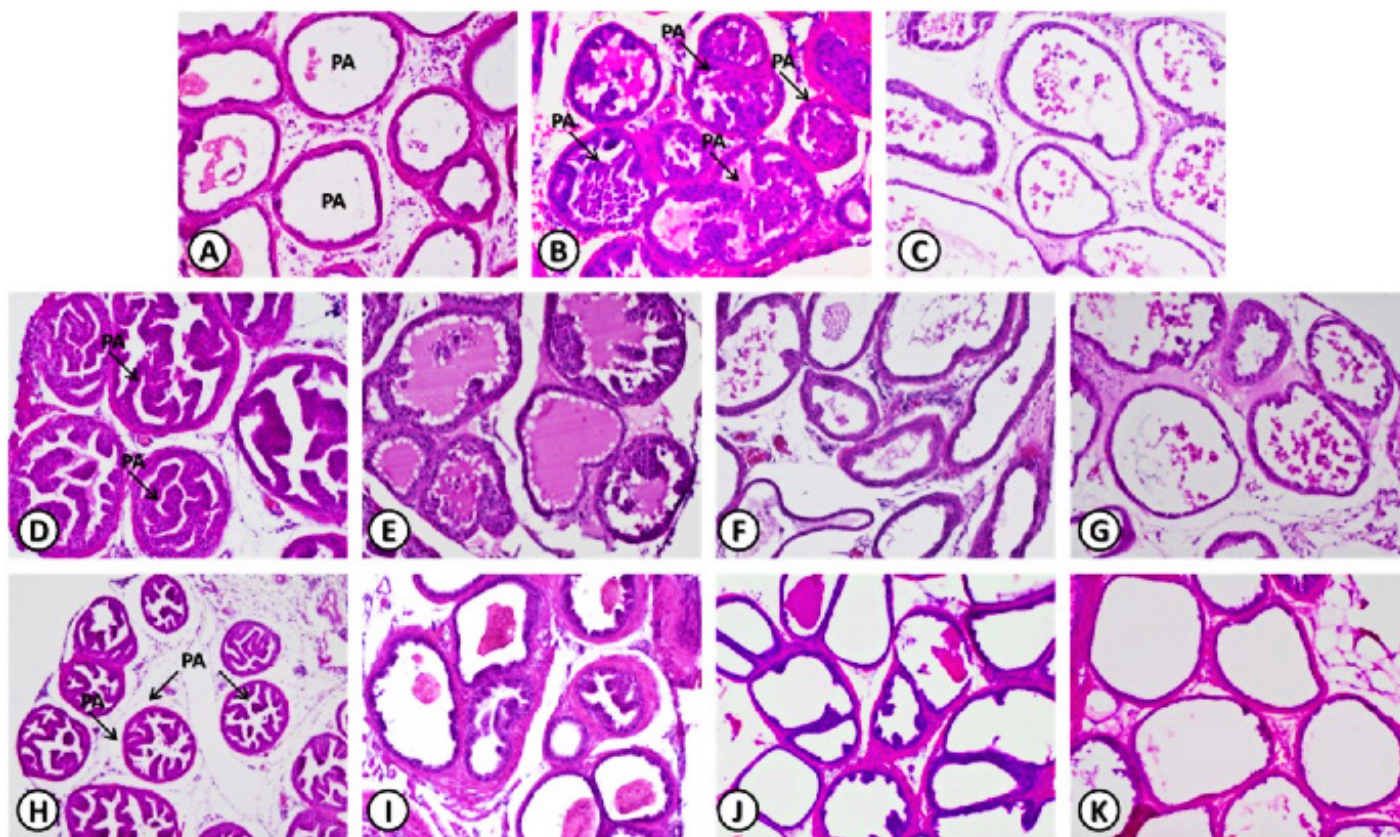
## DISCUSSION

Benign prostate hyperplasia (BPH) is non-malignant, enlargement and over proliferation of the stromal and glandular epithelial cells of the prostate tissue. Clinically BPH is associated with lower urinary tract symptom (LUTS) relating possibly to benign prostate enlargement (BPE) and benign prostate obstruction (BPO) that can be significant impact on the quality of

life of the patient.<sup>[19]</sup> Testosterone and dihydrotestosterone (DHT) is the major gonadal androgen hormone and are commonly associated with BPH.<sup>[20]</sup> In the present study, BPH in mice model was successfully induced by subcutaneous administration of testosterone (5 mg/kg) injection as observed in the day dependent enlargement of the prostate (Table 1). The prostate enlargement is used as an important marker indicating the development of BPH.<sup>[16,21]</sup> Testosterone (5 mg/kg) treated mice in day dependent manner showed significant increase in prostate weight and prostatic index when compared with negative control group and prostatic hyperplasia was also observed during histopathological examinations (Figure 1). No significant difference in body weight was found in different experimental group.

Currently two conventional therapeutic agents are available for treating BPH such as α-adrenergic blockers like doxazosin, terazosin and tamsulosin which help in relaxation of smooth muscle in the prostate and bladder neck to improve urinary flow and 5α-reductase inhibitors such as finasteride and dutasteride are used for the treatment of BPH.<sup>[18,22,23]</sup> However, long term use of these drugs leads to unpleasant side effects like erectile dysfunction, loss of libido, dizziness, upper respiratory tract infection, gynecomastia, impotence, chest pain etc its use is restricted.<sup>[24,25]</sup> Therefore phytotherapeutic and pharmaceutical agents of plant origin have proven to be an effective treatment option in BPH patients with low cost and minimal side effects.<sup>[26,27]</sup>

Several bioactive component like kaempferol, quercetin, β-sitosterol, oleic acid, palmitic and linoleic acid<sup>[7,28]</sup> have been identified from *Ageratum conyzoides* L. plant. These are reported to have the potential to inhibit BPH and prostate cancer by the activity of anti-cancer, antioxidant, 5α-reductase inhibitory effect, anti-proliferative, anti-inflammatory, anti-androgenic and apoptotic activity.<sup>[29-31]</sup> Recent studies also show that ethylalcohol extract of the aerial parts of *Ageratum conyzoides* L. was effective in reducing the expression of mRNA coding for 5α-reductase type 2 and 1 in *in-vitro* human prostate epithelial cell.<sup>[32]</sup>



**Figure 3:** Prostate histomorphology after administration of aqueous and ethanolic plant extracts of *Ageratum conyzoides* L. in testosterone (5mg/kg/day, S.C) induced BPH in mice model. (A) Negative control group; (B) BPH model or positive control group; (C) Finasteride (5mg/kg/day); (D-G)  $AC_{\text{aqueous}}$  plant extract treated group (100, 500, 1000 and 2000 mg/kg/day); (H-K)  $AC_{\text{ethanol}}$  plant extract treated group (100, 500, 1000 and 2000 mg/kg/day). H&E Stain, X400 magnifications.

In the prostate cell, testosterone can be converted to dihydrotestosterone (DHT) by the enzyme 5 $\alpha$ -reductase. DHT has 5 times higher affinity for the androgen receptor in the nucleus than testosterone and excessive production of DHT with increasing aging may play a critical role in the pathogenesis of BPH. The 5 $\alpha$ -reductase inhibitors for example, finasteride and dutasteride inhibit the development of BPH via a reduction in dihydrotestosterone (DHT) concentration,<sup>[33]</sup> resulting in the reduction of prostate size and BPH related lower urinary tract symptom [LUTS, 20]. However long-term use of finasteride produce serious side effects,<sup>[34]</sup> which has led researcher to investigate the alternative use of phytotherapy for BPH treatment with lower adverse effect. Therefore we evaluated dose dependent inhibitory effect of aqueous and ethanolic plant extract of *Ageratum conyzoides* L. on testosterone induced benign prostatic hyperplasia (BPH) in mice. In the current study, animal with BPH induced by testosterone treated mice showed significant increase in prostate weight and prostatic index when compared with the negative control group and prostatic hyperplasia with both stromal proliferation and glandular hyperplasia was also observed during histopathological analysis of the prostate tissue. In contrast, *Ageratum conyzoides* L. plant extract treated mice significantly and dose dependently inhibited the increased in prostate weight and prostatic index induced by testosterone in

mice model and had similar effect to finasteride, a standard drug currently used to treat BPH. Histopathological examinations also showed that treatment with aqueous and ethanolic plant extract of *Ageratum conyzoides* L. significantly ameliorated the increased in glandular hyperplasia and also reduced epithelial thickness and stromal space when compared to the BPH induced group.

Comparison of percentage inhibition exhibited by both plant extracts was found to be dose dependent and ED<sub>50</sub> calculation revealed that  $AC_{\text{ethanol}}$  plant extract (156.31 $\mu\text{g}/\mu\text{l}$ ) is slightly more effective than  $AC_{\text{aqueous}}$  plant extract (241.81 $\mu\text{g}/\mu\text{l}$ ) in the management of testosterone induced BPH. The dose of both plant extracts selected in the present experiment didn't cause any significant elevation in serum ALT and creatinine level, which indicated that these doses don't cause any hepatic and kidney toxicity in the experimental animals.

Phytotherapy with various plant extracts are being increasingly used in the treatment of benign prostatic hyperplasia for over decades because of its mildness, effectiveness and low adverse effects. The common phytopharmaceutical agents include *Brassica alba*, *Brassica napus* L., *Cocos nucifera* L., *Cucurbita pepo* L., *Serenoa repen*, *Lauric and myristic acid*, *Pygeum africanum* extract, *Urtica dioica* L. etc which protect prostate hyperplasia via their antioxidant, 5 $\alpha$ -reductase inhibitory effect,

**Table 3: Dose response study of AC<sub>Aqueous</sub> and AC<sub>EthOH</sub> plant extracts of *Ageratum conyzoides* L.**

Groups	Absolute Liver weight (g)	Relative Liver weight (g)	Serum ALT (U/ml)	Absolute Kidney weight (g)	Relative Kidney weight (g)	Serum Creatinine level (mg %)	Testes weight (g)	Testicular Index
Negative Control	1.52 ± 0.13	4.51 ± 0.26	21.27 ± 2.15	0.54 ± 0.04	1.61 ± 0.08	1.03 ± 0.23	0.21 ± 0.01	0.64 ± 0.04
Positive Control (T 5mg/kg)	1.69 ± 0.16	4.89 ± 0.36	24.60 ± 0.97	0.52 ± 0.03	1.51 ± 0.06	1.19 ± 0.20	0.16 ± 0.03	0.46 ± 0.09
T + Finasteride (3mg/kg)	1.70 ± 0.07	5.72 ± 0.12	34.48 ± 8.55	0.57 ± 0.03	1.92 ± 0.06*	0.54 ± 0.16	0.18 ± 0.01	0.62 ± 0.06
<b>AC<sub>Aqueous</sub></b>								
T + AC <sub>Aqueous</sub> (100mg/kg)	1.68 ± 0.15	4.91 ± 0.18	16.17 ± 0.55	0.50 ± 0.03	1.48 ± 0.06	0.91 ± 0.11	0.20 ± 0.01	0.60 ± 0.03
T + AC <sub>Aqueous</sub> (500mg/kg)	1.45 ± 0.13	4.10 ± 0.22	22.72 ± 2.71	0.52 ± 0.05	1.47 ± 0.09	1.27 ± 0.15	0.16 ± 0.02	0.46 ± 0.09
T + AC <sub>Aqueous</sub> (1000mg/kg)	1.80 ± 0.15	5.57 ± 0.25	20.48 ± 2.50	0.54 ± 0.04	1.69 ± 0.09	0.70 ± 0.06	0.20 ± 0.01	0.62 ± 0.03
T + AC <sub>Aqueous</sub> (2000mg/kg)	2.21 ± 0.07	6.68 ± 0.08***	28.42 ± 3.14	0.49 ± 0.02	1.49 ± 0.04	0.57 ± 0.06	0.22 ± 0.00	0.66 ± 0.04
<b>AC<sub>EthOH</sub></b>								
T + AC <sub>EthOH</sub> (100mg/kg)	1.44 ± 0.11	4.33 ± 0.38	29.21 ± 0.97	0.59 ± 0.02	1.76 ± 0.07	0.74 ± 0.30	0.23 ± 0.01	0.70 ± 0.05
T + AC <sub>EthOH</sub> (500mg/kg)	1.56 ± 0.15	4.46 ± 0.28	17.57 ± 0.78	0.62 ± 0.05	1.77 ± 0.08	1.49 ± 0.06	0.19 ± 0.02	0.57 ± 0.09
T + AC <sub>EthOH</sub> (1000mg/kg)	1.52 ± 0.12	4.81 ± 0.25	25.15 ± 5.34	0.52 ± 0.03	1.66 ± 0.13	0.50 ± 0.02	0.16 ± 0.03	0.52 ± 0.10
T + AC <sub>EthOH</sub> (2000mg/kg)	1.79 ± 0.07	5.39 ± 0.24	26.27 ± 3.83	0.52 ± 0.02	1.57 ± 0.06	0.68 ± 0.08	0.22 ± 0.01	0.67 ± 0.03

On liver, kidney and testis weight, serum ALT and creatinine level, testicular index in testosterone induced BPH in mice model. Values are expressed as Mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Newman Keuls multiple comparison test, \* $p < 0.05$  and \*\*\* $p < 0.001$  when compared BPH model.

anti-proliferative, anti-inflammatory and anti-androgenic activity due to presence of their active compounds like kaempferol, quercetin, sinabin,  $\beta$ -sitosterol, oleic acid, palmitic and linoleic acid, lauric and myristic acid, polysaccharides etc responsible for their BPH inhibitory activity.<sup>[26,27]</sup> *Ageratum conyzoides* L. plant also contain kaempferol, quercetin,  $\beta$ -sitosterol, oleic acid, palmitic and linoleic acid.<sup>[7,28]</sup> Further it was reported that the ethyl alcohol extract of the aerial parts of *Ageratum conyzoides* L. was effective in reducing the expression of mRNA coding for 5 $\alpha$ -reductase type 2 and 1 in *in-vitro* human prostate epithelial cell.<sup>[32]</sup> Together this study provided sufficient information regarding the beneficial effect of *Ageratum conyzoides* L. plant extract on the testosterone induced BPH model.

## CONCLUSION

The aqueous and ethanolic plant extract of *Ageratum conyzoides* L. significantly decreases prostate weight, prostatic index and prostate hyperplasia in the testosterone induced BPH animal.

During present investigation it was observed that testosterone induced BPH which lead to cell proliferation was significantly attenuated as shown in increase in percentage of inhibition caused by AC plant extract treatment. In addition histopathological observation have shown that with AC plant extract treatment in mice model significantly reduced the increased in glandular hyperplasia, epithelial thickness and stromal space. Also it did not affect the normal function of the liver and kidney. Hence, this finding indicated that *Ageratum conyzoides* L. has potential inhibitory effect in testosterone induced BPH in mice model. Looking at its inhibitory properties on the BPH model, further studies are necessary to analyze active compounds and identify its anti-proliferative properties in prostatic hyperplasia.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**BPH:** Benign Prostate Hyperplasia; **ALT:** Alanine Aminotransferase; **AC:** *Ageratum conyzoides*; **LUTS:** Lower Urinary Tract Symptoms; **DHT:** Dihydrotestosterone; **TP:** Testosterone Propionate; **PI:** Prostate Index; **NC:** Negative Control; **PC:** Positive Control; **T:** Treated group; **SC:** Subcutaneous; **ED<sub>50</sub>:** Effective Dose 50; **BPO:** Benign Prostate Obstruction.

## SUMMARY

Benign prostate hyperplasia (BPH) is non-malignant progressive, enlargement and over proliferation of the stromal and glandular epithelial cells of the prostate tissue. Conventional therapeutic agents used to treat BPH have various side effects. Therefore phytotherapeutic and pharmaceutical agents of plant origin have proven to be an effective treatment with low cost and minimal side effect. *Ageratum conyzoides* L. which is commonly used for its medicinal, pharmacological and therapeutic properties have showed the aqueous and ethanolic plant extract has potential inhibitory effect on testosterone induced BPH in mice model.

## CONTRIBUTIONS

Tongchen Jomba: Carried out the experiment, data collection and drafting of the manuscript. Manuj Kr Bharali: Conceived and designed the study, data analysis and finalization of the manuscript.

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