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# Inhibitory effect of *Dillenia indica* L. bark extract on testosterone induced benign prostatic hyperplasia in rat

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a common urological disorder of elderly men, which is characterized by hyperplasia of prostatic stromal and epithelial cells. The present study was designed to investigate the inhibitory effect of the Aqueous Bark Extract of *Dillenia indica* L. (*ABEDI*) in the BPH rat model. The male rats were treated either corn oil or testosterone (10 mg/kg) dissolved in corn oil and testosterone with *ABEDI* (100 and 500 mg/kg) consecutively for three weeks. The inhibitory effect of *ABEDI* on BPH was illustrated by prostate weight, prostatic index, prostate epithelial height, percentage of inhibition and histological examinations. *ABEDI* caused significant reduction in the prostate weight and prostatic index compared to testosterone induced BPH model group. Serum ALT and creatinine levels did not differ among different experimental groups, when compared with a positive control group. *ABEDI* treated group also ameliorated the hyperplasia of prostate epithelium in a similar manner as observed in the finasteride (5 mg/kg) treated group. This study indicates that *ABEDI* significantly reduced the progression of BPH and it may be another phytotherapeutial source of drugs in BPH treatment.

KEYWORDS: *Dillenia indica* L., BPH, bark extract, testosterone, and rat.

## Introduction

Benign prostatic hyperplasia (BPH) is an uncontrolled proliferation of the both epithelial and stromal cells that occurs in the transitional zone of the prostate gland. Clinically, it is characterized by presenting lower urinary tract symptoms (LUTS), nocturia, sepsis, irreversible bladder failure, and even death (Arruzabala et al., 2007; Pais, 2010). BPH is one of the common prostate problems experienced by around 85% of men above the age group of 45 years (Sarma and Wei, 2012).

Although the etiology of BPH is not clearly understood, but sex hormone (androgen) is known to play a significant role in the disease progression. The development and growth of prostate gland depend on androgen stimulation, mainly by dihydrotestosterone (DHT), an active metabolite formed due to enzymatic conversion of testosterone by 5 $\alpha$-reductase (Carson and Rittmaster, 2003). Production and accumulation of DHT in the prostate gland increases with aging, which results in cell proliferation and induction of prostatic hyperplasia (Carson and Rittmaster, 2003; Bartsch

et al., 2000). Conventionally used drugs like 5 $\alpha$-reductase inhibitors (finasteride and dutasteride), $\alpha$-adrenoceptor antagonists (alfuzosin, doxazosin, tamsulosin, terazosin) are used for the management of BPH (Clark et al., 2004). But these drugs possess adverse side effects like impotence, decreased libido, ejaculation disorder, gynaecomastia, dizziness, upper respiratory tract infection, headache, fatigue etc. (Bullock and Andriole, 2006). Alternate therapy like phytotherapy has been proven to be an effective treatment options in BPH patients without or minimal side effects.

The plant *Dillenia indica* L. (Dilleniaceae) is a frequently reported traditional medicinal plant known to possess antihyperlipidemic (Kumar et al., 2011), anti-leukemic (Kumar et al., 2010), and antioxidant activity (Deepa and Jena, 2011). Traditionally, the juices of leave bark and fruits of *Dillenia indica* L. is used for the treatment of cancer in the tribal areas of Northeast, India (Rosangkima et al., 2010; Singh et al., 2016). The plant is reported to contain betulinic acid, betulin, dillenetin, cycloartenone and

β-sitosterol (Muhit et al., 2010). Among all of them betulinic acid contains highest anti-cancer property (Hsu et al., 2015; Xu et al., 2017). Till today, no study has been tested the efficacy of *Dillenia indica* L. on a testosterone induced animal BPH model. Hence, by taking this information into account, the present study has been undertaken to investigate the inhibitory effect of the aqueous bark extract of *Dillenia indica* L. (*ABEDI*) on the BPH rat model.

## Materials and methods

### Chemicals
Testosterone, corn oil (Sigma-Aldrich) and Finasteride (FINAST, Dr. Reddy's Laboratories) were used. All other chemicals used during the experiment were of analytical grade.

### Experimental plant
The experimental plant was collected from the Rajiv Gandhi University campus and identified with the help of Dr. Hui Tag, Associate Professor, Department of Botany, Rajiv Gandhi University. The voucher specimen (LBC/RGU/2013/02) was deposited at the Centre with Potential for Excellence in Biodiversity (CPEB), Rajiv Gandhi University for future reference. The fresh stem bark of *Dillenia indica* L. was washed, shade dried, powdered. 5 g of powder was dissolved in 100 ml double distilled water for overnight, filtered and kept at -20°C for further use.

### Experimental animals
Male Sprague-Dawley rats weighing 80-120 g were (Stock animal facility of the department of Zoology, Rajiv Gandhi University) kept in polycarbonate cages and rice husk were used as a bedding material with twelve hour light/dark cycle. Animals were provided with a standard laboratory diet and water *ad libitum*. The experiment was carried out in accordance with the NIH Guidelines (NIH, 1985) for the Care and Use of Laboratory Animals. All experimental procedures were examined and approved by the institutional animal ethical committee (No. IAEC/RGU/16/09).

### Induction of BPH and treatments
After one weeks of acclimatization in laboratory condition, animals (n=5) were randomly divided into five groups. Group I served as a negative control and received subcutaneous (s.c.) injection of corn oil only. BPH model was induced in experimental groups by daily subcutaneous injection of testosterone (10 mg/kg) dissolved in corn oil from day 0 to day 7 (induction phase). The dosage and

duration of testosterone treatment was determined by our previous report (Bharali and Chetry, 2014). After one week of BPH induction, animals were divided into four different experimental groups. Group II served as BPH model and continued testosterone (10 mg/kg) for the rest of the experimental period, Group III received daily intraperitoneal (i.p.) injections of 5α-reductase inhibitor, finasteride (5 mg/kg) along with testosterone. The dosage of finasteride was based on previous study (Fitts et al., 2004; Rudolfsson and Bergh, 2008). Group IV and V of animals were given daily intraperitoneal injections of *ABEDI* (100 and 500 mg/kg) along with testosterone. All animals were treated once daily for three consecutive weeks. During the experiment, no external morphological and behavioral changes were observed in the animals treated at these dose levels.

After the final treatment, the animals were deprived of food overnight, but were allowed free access to water. Body weights were measured at the onset of experiment and at the time of termination to measure the weight gain or loss during the study. In the morning of the next day, animals were weighed and then sacrificed by sacrificed by cervical dislocation using ketamine hydrochloride (10 mg/kg, i.p.) as anesthesia. Blood was collected from the cardiac puncture and allowed to clot and serum was separated at 10000 rmp for 15 min and stored at -20°C for further analysis.

### Calculation of prostatic index and percentage of inhibition
The prostatic index and the percentage inhibition of prostate enlargement were assessed by the Ali et al., 2013.

$$\text{Prostatic Index (PI)} = \frac{\text{Prostate Weight (g)}}{\text{Final Body Weight (g)}} \times 100$$

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

where T, NC and T represents the prostatic index values of the treatment group, negative control and positive control group of animal respectively.

### Histopathological examination
Whole prostate and liver were isolated and cleaned from fat tissue, weighed and fixed in 10% neutral buffered formalin for 24 h. After washed with distilled water, the prostate was dehydrate in a progressive series of alcohol (30-100%), cleared in xylene and embedded in paraffin. Transverse sections (5 μm) were stained with haematoxylin and eosin for histological study. Five microscope fields/mouse were photographed and analyzed in sections

from five mice for each group using Leica DM5000B ((Leica Microsystems, Germany) microscope. The prostate epithelial height (μm) was measured by manually drawing a line through the acinar epithelia (30 measures per field) by using Image J software (Developed at US National Institute of Health).

### Estimation of alanine aminotransferase (ALT) and creatinine levels in serum
Serum alanine aminotransferase (ALT) (Reitman and Frankel, 1975) and creatinine levels were estimated by using commercial kits (Coral system, Goa, India).

### Statistical analysis
All data were expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at $P < 0.05$.

## Results
### Effect of ABEDI on body and prostate weights
The administration of testosterone (10 mg/kg) significantly ([a]$P < 0.001$) increased the absolute body weight of all the animals of the BPH model group when compared to the negative control (Table 1). *ABEDI* (100 and 500mg/kg) administered group also showed the significant ([d]$P < 0.001$) increased with body weight along with testosterone (10 mg/kg) treatment when compared to the BPH model group (Table 1).

The increased prostatic weight is a vital marker for the progression of BPH. In the present study, significant (0.712 ± 0.0009; [a]$P < 0.001$) elevation in prostate weights was revealed by testosterone (10 mg/kg) treatment when compared with a negative control group (Table 1). At the same time, significant reduction in elevation of prostatic weights was found by the *ABEDI* 100 mg/kg (0.235 ± 0.007; [d]$P < 0.001$) and *ABEDI* 500 mg/kg (0.297 ± 0.004; [d]$P < 0.001$) administration when compared to the testosterone treated rats (Table 1). The finasteride treatment also caused a significant reduction in the absolute prostate weight (0.375 ± 0.009; [d]$P < 0.001$), when compared with the testosterone treated rats (Table 1).

### Effect of ABEDI on prostatic index and percentage of inhibition
The prostatic index is commonly used to evaluate the development of prostatic enlargement. The induction of BPH significantly ($P < 0.001$) increased the prostatic index in a testosterone induced BPH animals, as compared to the

negative control group. The treatment with finasteride (5 mg/kg) and *ABEDI* (100 and 500mg/kg) significantly decreased the prostatic index when compared to the testosterone treated BPH model group ($P < 0.001$) (Fig. 1). The percent of inhibition of prostate enlargement by standard drug finasteride and *ABEDI* 100 mg/kg was observed to be 66.98 and 68.60%, respectively; whereas the administration of *ABEDI* 500 mg/kg exhibited maximum inhibition of 75.25% (Table 1).

| Groups | Body weights (g) | | | Prostate weights (g) | % of Inhibition |
|---|---|---|---|---|---|
| | Initial | Final | Difference | | |
| I | 80 ± 3.65 | 102.5 ± 0.91 | 22.5 ± 4.56 | 0.174 ± 0.018 | - |
| II | 131 ± 4.01• | 152.87 ± 4.51• | 21.87 ± 0.50 | 0.712 ± 0.0009• | - |
| III | 118.15 ± 0.67• | 139.67 ± 0.11• | 21.52 ± 0.79 | 0.375 ± 0.009• | 66.98 |
| IV | 73.72 ± 0.08• | 89.34 ± 0.71• | 15.62 ± 0.79 | 0.235 ± 0.007• | 68.60 |
| V | 97 ± 2.55• | 121.79 ± 1.62• | 24.79 ± 0.92 | 0.297 ± 0.004• | 75.25 |

**Table 1.** Effect of *ABEDI* (Aqueous bark extract of *Dillenia indica* L.) on body weights and prostate weights in testosterone treated rats.

Group I: Negative control, Group II: BPH model (Testosterone 10 mg/kg), Group III: Testosterone 10 mg/kg + Finasteride 5 mg/kg), Group IV and V: Testosterone 10 mg/kg + *ABEDI* (Aqueous bark extract of *Dillenia indica* L.) 100 and 500 mg/kg respectively. Values are expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at $P < 0.05$, ·$P < 0.05$ when compared with the II group, ·$P < 0.01$ when compared with the II group, ·$P < 0.001$ when compared with the I group, ·$P < 0.001$ when compared with the II group.
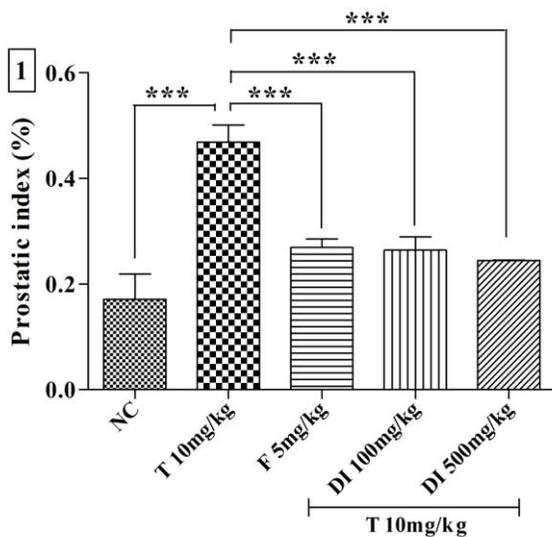
### Effect of ABEDI on histopathological examination of prostate and prostatic epithelial heights
There was no much change in the histoarchitecture of the prostate gland in the negative control group. The tissues were tightly packed; epithelium was cuboidal and regular in size (Fig. 2 A). In the BPH model group irregular acinar folding with intraluminar projections were observed. The amount of connective tissue was well marked with increase oval acini size. The stromal proliferation and glandular hyperplasia with epithelial proliferation and nuclear stratification have been observed (Fig. 2 B). The administration of a 5α reductase inhibitor finasteride has found to inhibit epithelial hyperplasia in the prostate acini (Fig. 2 C). Treatment with *ABEDI* (100 and 500 mg/kg) reduces testosterone induced glandular hyperplasia and epithelial proliferation as evidence by decreased in the number of intraluminal projections (Fig. 2 D and E).

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BPH induction showed a significant change in the epithelial height of the prostate gland of the BPH model group (*P*<0.001) when compared with the normal control group (Fig. 3). However, the treatment with *ABEDI* (100 and 500 mg/kg) significantly (*P*<0.001) decreased the epithelial height when compared with the BPH model group (Fig. 3). Administration of 5 α reductase inhibitor finasteride (5 mg/kg) also significantly reduced the epithelial height in the prostate acinar cells (*P*<0.001; Fig. 3). The above findings indicate the marked restoration of disrupted histoarchitecture by *ABEDI* (100 and 500 mg/kg) when compared with a BPH model group.
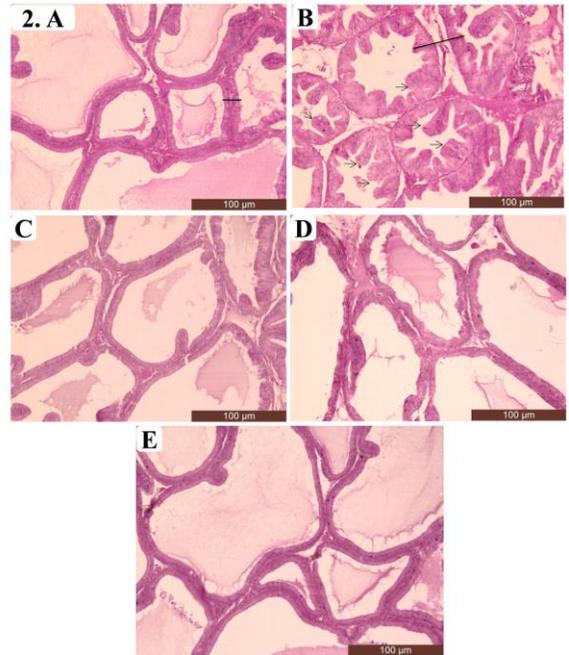
*Toxicity of ABEDI in a rat model of BPH*

In order to evaluate toxicity in the liver and kidney, serum ALT and creatinine were tested. The ALT and creatinine levels in serum were not significantly different among all the experimental groups (Fig. 5 and 6). The administration of *ABEDI* (100 and 500mg/kg) did not promote the activity of the serum toxicity marker enzyme, such as ALT in the liver and creatinine in the kidney. Similarly, the treatment of *ABEDI* (100 and 500mg/kg) did not alter any changes in histoarchitecture of the liver in all the experimental groups (Fig. 4). Thus, it is confirmed that the animals of each group had a normal function of livers and kidneys.
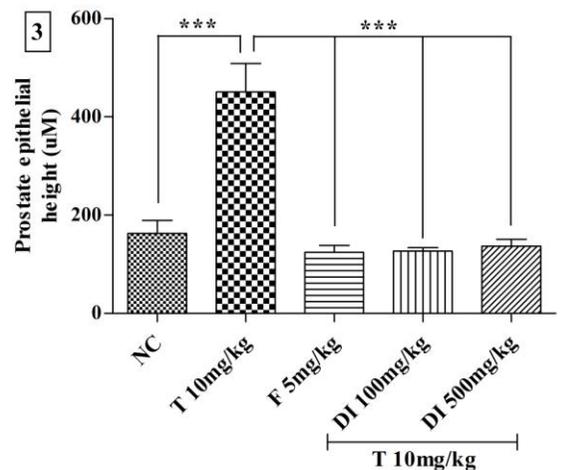
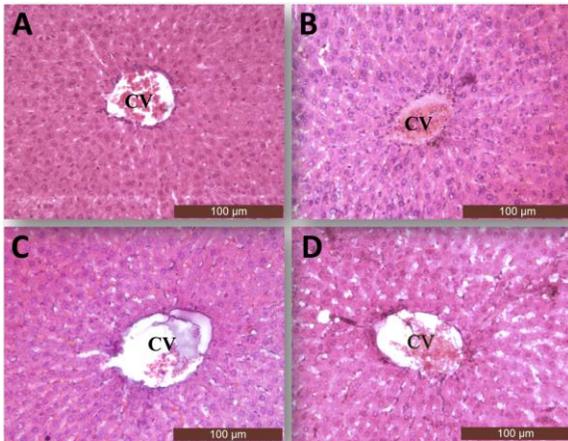<0.001 when compared to positive control group (BPH model group).



Figure 2. Representative photomicrograph of rat prostate tissues of different experimental groups. **A.** Negative control, **B.** Positive control (Testosterone 10 mg/kg), **C.** Finasteride 5mg/kg + T 10 mg/kg, **D.** *ABEDI* 100 mg/kg + T 10 mg/kg, **E.** *ABEDI* 500 mg/kg + T 10 mg/kg. HE: Hematoxylin and Eosin stain; magnification 20X; Leica DM5000B. Straight line represents epithelial height of prostate.
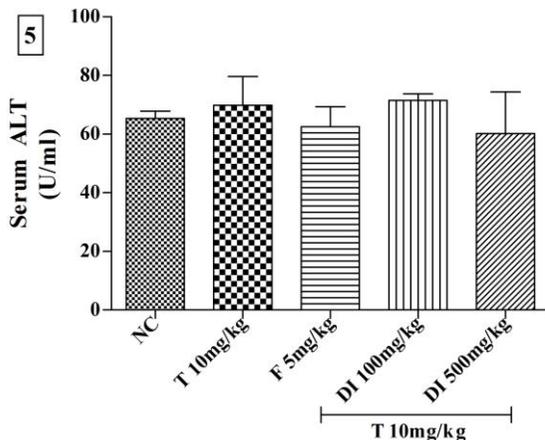


Figure 1. Effect of *ABEDI* on prostatic indices in different experimental groups. Values are expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at *P*< 0.05. ***P



Figure 3. Effect of *ABEDI* on prostatic epithelial heights in different experimental groups. Values are expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at *P*< 0.05.

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***$P$<0.001 when compared to positive control group (BPH model group).



**Figure 4.** Representatives of histological features of liver from different experimental groups. **A.** Negative control, **B.** Positive control (Testosterone 10 mg/kg), **C.** Finasteride 5mg/kg + T 10 mg/kg, **D.** and **E.** *ABEDI* 100 and 500 mg/kg + T 10 mg/kg. HE: Hematoxylin and Eosin stain; magnification 10X; Leica DM5000B.
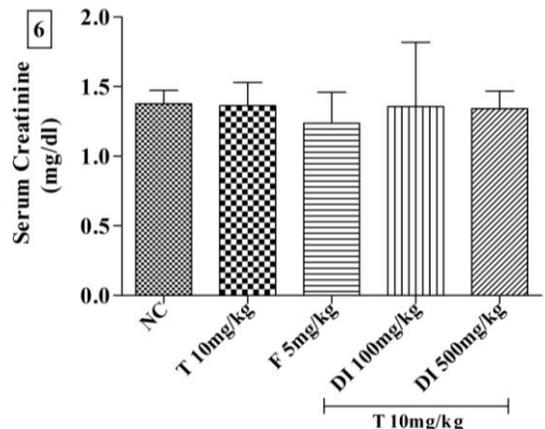


**Figure 5.** Effect of *ABEDI* on Serum ALT level in different experimental groups. No statistical significant was found in serum marker of liver injury (ALT) in any groups during the experimental period. Values are expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at $P<$ 0.05.

## Discussion

In this present investigation, the administration with *ABED* (Aqueous bark extract of *Dillenia indica* L.) (100 and 500mg/kg) for three consecutive weeks significantly inhibited the development of testosterone induced prostatic hyperplasia, which was evidenced by the reduction in

elevated prostate weight and prostatic index and histopathological studies.

It has been established that steroid, 5 α-reductase enzymes abundantly found in the nuclear membrane microsomes of prostatic epithelial cells, and is involved in the conversion of testosterone to its active metabolite called dihydrotestosterone (DHT). An elevated level of dihydrotestosterone in prostate gland results in the development of BPH (Carson and Rittmaster, 2003). Conventional drugs like 5 α-reductase inhibitors reduce the concentration tissue DHT without interfering with the sexual function by blocking the formation of DHT (Clark et al., 2004).



**Figure 6.** Effect of *ABEDI* on serum creatinine level in different experimental groups. No significant alteration in serum marker of renal injury (Creatinine) observed in any groups during the experimental period. Values are expressed as mean ± SEM (n=5). Statistical analysis is done by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level for significance was set at $P<$ 0.05.

The prostate enlargement is a vital marker for the experimental evaluation of BPH (Veeresh Babu et al., 2010; Pais, 2010). In the present study, the testosterone treated group revealed a significant increased in body weight, prostate weight and prostatic index as compared to the negative control group. A 5 α-reductase inhibitor finasteride treatment group also evidenced the lower prostate weight and prostatic index as compared to the testosterone treated BPH model group. In contrast, treatment with *ABEDI* (100 and 500mg/kg) showed a significant reduction in prostate weight compared to the

BPH model group. These results were reliable with histopathological examinations of prostatic tissues. Several earlier studies also reported the effectiveness of alternative and complementary therapy of plant origin, such as *Serenoa repens* (Paubert-Braquet et al., 1996), *Semen vaccariae* (Zhang et al., 2013), *Cucurbita pepo* (Nawfal et al., 2011), *Urtica dioica* (Nahata and Dixit, 2011), *Boerhaavia diffusa* (Vyas et al., 2013) and many more. The findings of the present investigation supported the promotion of phytotherapy as demonstrated by earlier workers in the management of BPH in experimental animals.

Liver is a major site for the detoxifications of various toxicants and drugs (Ahsan et al., 2009). ALT (alanine aminotransferase) has been commonly used as a diagnostic marker for hepatoxicity (Pumford et al., 1997). When a liver cell is damaged or diseased from drugs or toxic substance the ALT released into the blood circulation, which makes ALT level rise up. In this present study treatment of *ABEDI* (100 and 500mg/kg) along with the testosterone treatment did not showed any significant difference in all experimental animals. Thus, it indicates the normal function of the liver, which is supported by previous reports (Reddy et al., 2010).

The serum creatinine is vital biomarkers for the renal dysfunction. Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscles. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunctions (Lopez-Giacoman and Madero, 2015). In this study, the serum creatinine was determined in all the groups and did not show any significant difference in all experimental animals.

Together, this study provided sufficient information regarding the beneficial effect of the aqueous bark extract of *Dillenia indica* L. on the testosterone induced BPH model.

## Conclusion

In conclusion, intraperitoneal administration of *ABEDI* (100 and 500 mg/kg) in a testosterone induced BPH model animals significantly decreased the prostate size, prostatic index, prostatic epithelial height and prostate hyperplasia respectively. The present study has also shown that serum ALT and creatinine, potential biomarkers of liver and renal injury remains unaltered by continuous administration of *ABED* for 21 days. Histopathological examination of the

liver also confirmed that *ABED* had no adverse effect on the liver at selected doses. These findings indicate that *ABEDI* may effectively decrease the progression of the testosterone induced BPH model. Altogether, this study firmly suggests that the *ABEDI* possess active chemical components which may be important for futures potential of drug discovery for effective treatment for BPH.

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