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RESEARCH ARTICLE

Anti-BPH Activity of Polyherbal Formulation on Testosterone Induced Benign Prostatic Hyperplasia in Rats

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Abstract

Benign prostatic hyperplasia (BPH) is an enlargement of the prostate gland caused by progressive hyperplasia, or abnormal growth of cells of the glandular epithelial and stromal cells. Globally, it has been documented that more than 80% of men by the age of 80 will suffer from BPH. Most men are hesitant to undergo surgical interventions for fear of losing potency and the perception of other adverse side effects. Effectto® is a polyherbal formulation designed by Vasu Research Center to be used for the treatment of BPH. It can also be indicated that it can be used for lower urinary tract symptoms and bladder outlet obstruction. The formulation is expected to provide significant BPH relief. The present study was planned to evaluate the effect of a polyherbal formulation on testosterone- and citral-induced Prostatic Hyperplasia. The activity of the polyherbal formulation for BPH was evaluated using testosterone depot injection as the inducing agent in a testosterone-induced model and citral-induced model for the atypical type of BPH in rats. Also, the acute toxicity study was done using OECD 423 guidelines to check the toxicity of the test compound. Data are expressed as mean ± SD and statistical significance was evaluated using one way ANOVA followed by Tukey's multiple comparison tests. The polyherbal formulation was found to be safe at oral doses of 2000 mg/kg. Effectto® significantly decreased the weight of the prostate in the testosterone model as well as the citral model in rats. The effect on biochemical markers like serum PSA and TNF-α was also seen in both the cases. In particular serum PSA, the decrease was majorly significant in both the models when XIII compared. The formulation was able to reverse the effect of inducing agents on the prostate's size.

Keywords: Benign Prostatic Hyperplasia, Citral, Effectto®, Finasteride, LUTS, PSA, Testosterone

1. Introduction

Benign Prostatic Hyperplasia or BPH is a medical term used to describe a change in the histology of the prostate cells in males. This change leads to prostate enlargement. This prostate condition is very often seen in old men. This BPH causes other conditions like Bladder Prostatic Obstruction (BPO) which is defined as an obstructed outflow, i.e. blocking the urine outflow in the urethra. Another term associated with BPH is Lower Urinary Tract Symptoms (LUTS) which involve organs like the prostate, urinary bladder and urethra. The LUTS increases in parallel with age[1].

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The Lower Urinary Tract Symptoms associated with BPH seen in older men includes:

- Urgency of urination
- Frequent urination
- Nocturia
- Decreased and intermittent force of the stream
- Sensation of incomplete bladder emptying[2]

BPH symptoms include difficulties related to storage and voiding, which are the two major symptoms. Symptoms that are linked to storage include changes in urinary frequency, urgency, involuntary urination, and excessive urination at night. Other voiding-related symptoms include urinary stream, hesitancy, intermittency, straining to void, and dribbling[3].

Tens of millions of men around the world are affected by BPH and BOO. In countries like the US, Europe and others, the current disease trends suggest that the incidence and prevalence of these conditions will increase due to factors like the ageing of the world population and an increase in the prevalence of metabolic syndromes. Age and genetics play their role in the BPH and BOO, there are other modifiable variables too that play their role in it. 40 % of men who are aged 50-60 years and 90% of men aged 80-90 years are suffering from BPH. The cause of BPH is still not defined. Studies says that there are causative factors like age, diet, lifestyle changes and hormonal imbalance that might have an effect on a wide range of older men, leading to prostate enlargement. Recent reports have suggested that there might be a strong relationship between metabolic syndrome and erectile dysfunction with prostate enlargement[4,5].

Causative factors likely to be found in BPH patients include:

- Age
- Genetic factors/Family history
- Medical conditions like obesity and circulatory diseases
- Lifestyle
- Erectile Dysfunction[4]

For the growth and development of the prostate gland, androgens are an important part of its physiology. DHT (dihydrotestosterone) and testosterone both have a significant effect on BPH growth. DHT is an endogenous androgen formed from testosterone. DHT has a three-fold greater affinity for androgen receptors. With ageing, an increase in DHT leads to intensifying the severity of BPH[6].The diagnosis of BPH/LUTS starts with obligatory evaluation of medical history, assess symptoms and scoring. Scoring is done using the I-PSS given by the AUA (American Urological Association). Physical examination is done using Digital Rectal Examination (DRE) and urinalysis is also included. Additional evaluation is needed to differentiate prostate cancer which includes finding Prostate Specific Antigen (PSA) concentration, ultrasound, and endoscopy and flow studies as well[7]. To check the therapeutic action of the test, several animal models have been used over the years. Animals like mice, rat and canine animals are used for BPH models. Though the aetiology of the disease is not defined yet the models are used several times over the decade for symptomatic relief[4]. The management of BPH should easily cause an elevation in the Quality Of Life (QOL). Special combinations and therapies are currently available that can improve the effects of BPH[8].

Adenosis of the prostate, also known as atypical hyperplasia (non-cancerous), is a proliferative lesion that causes an atypical type of BPH or also known as Atypical Prostatic Hyperplasia (APH). It can be observed in the epithelial cells of the transurethral section of the gland. Due to its architectural features, it mimics the adenocarcinoma of the gland. This may or may not have an effect on prostate size, but it does affect the histology of the cells[9].

Effectto® is a polyherbal formulation developed by Vasu Research Center to be used for the treatment of Benign Prostatic Hyperplasia (BPH). It can also be indicated that it can be used for Lower Urinary Tract Symptoms (LUTS) and for Bladder Outlet Obstruction (BOO). The ingredients that are included in the formulation have been described in traditional literature or studied individually and have been found to have anti-inflammatory, anti-proliferative, which may contribute to the positive anti-BPH effects of the formulation. Thus, the formulation is expected to produce a significant relief in BPH. However, there is no scientific preclinical data in evidence of its beneficial effects. Further, its safety and efficacy need to be evaluated using different models of Prostatic Hyperplasia. These data are also a prerequisite to clinical studies.

2. Material and Methods

Effectto® is a capsule of polyherbal formulation, and its ingredients are as shown in Table 1.

Table 1. Ingredients of Effectto® capsule

Sr. No.	Ingredients	Part used
	Extract of	
1	*Serenoa repens* (Saw palmetto)	Fruit
2	*Crateaeva nurvela* (Varun)	Bark
3	*Boerhaavia diffusa* (Punarnava)	Root
4	*Bauhinia variegata* (Kanchanar)	Bark
	Powder of	
5	Triphala churna	Quantity sufficient
6	Excipients	Quantity sufficient

2.1 Animals Used in the Study

Healthy male Wistar rats from 8 to 12 weeks were used for the study.

2.2 Acute Toxicity Test[10]

The acute oral toxicity study of Effectto® has been performed using the OECD 423 guideline – Toxic Class Method. The mortality related to the test compound determined the next steps of the procedure, i.e.,

- No further testing will be needed.
- Three additional animals will be given the same doses.
- Three more animals at the next higher, next higher or next lower dose level.

2.3 Dose

5, 50, 300 and 2000 mg/kg are the doses used for step-and-step procedures. A dose of equivalent to 2000mg/kg was made of the test substance, which is considered to be non-toxic. This dose was given on the first step of the limit test.

2.4 Testosterone Induced BPH Model in Rats[11,12]

Thirty Wistar male rats were randomly divided into five experimental groups of six rats each (Table 2). They were fed a standard laboratory diet and water ad libitum. 12 h dark-light cycle was maintained. Testosterone in olive oil was administered by subcutaneous injection for 28 days. The polyherbal formulation was suspended in 1% CMC solution and administered by oral gavage for 28 days.

Table 2. Grouping of animals

Group No.	Group	Treatment
1	Vehicle control group (Arachis oil)	1 mg/kg/day
2	Disease control group (Testoviron depot injection)	2.5 mg/kg/day (s.c)
3	Effectto® (Low dose)	90 mg/kg
4	Effectto® (High dose)	180 mg/kg
5	Standard drug control group (Finasteride)	1mg/kg/day (p.o)

2.5 Physical Parameter: Body Weight, Prostate Weight Index, Testis Weight Index was Observed

The weights of individual animals were recorded weekly from the day of randomization untill the end of the study. After the dissection of rats, the prostate and testis were isolated, washed in 0.9% saline solution, dried and their weight was measured. Then, in a 10% neutral formalin solution, the organ was fixed for histopathology.

2.6 Kidney Markers

Blood Urea Nitrogen (BUN) and serum creatinine were determined at the end of the study using a kit.

2.7 Biochemical Marker

Prostate Specific Antigen (PSA) and levels of TNF-α: were measured by the Instruction Manual, the quantitative determination of Rat PSA concentration[13,14].

2.8 Statistical Analysis

The results of the study were expressed as mean ± SD and were analyzed by one way A NOVA multiple comparison using Graph Pad Prism Software, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Acute Toxicity Study

Acute oral toxicity testing for the Effectto® capsules was done using OECD TG 423 in rats at a limit dose of 2000 mg/kg in three rats. No significant morbidity or mortality was observed in these rats and hence, a confirmatory study was taken up in three more rats. After the study,

there was still no mortality or significant side effects seen (Table 3). Hence, the formulation was considered safe at doses up to 2000 mg/kg.

Table 3. Observations for toxic class method

Parameters	Result
Body weight	No significant change
Clinical signs	No significant abnormalities
Behavioural change	No change
Autonomic symptoms	Normal
General awareness	Normal
Gross pathology	No abnormalities observed

3.2 Body Weight

The Body weights of all the animals were measured every week untill the termination of the study. A gradual increase in body weight was observed for all the groups. However, the increase in body weight in animals receiving testosterone at the end of the 28-day period was significantly greater ($p<0.05$) than that of the control group. Only Finasteride significantly ($p<0.05$) reversed testosterone's anabolic effect. The increase in body weight in animals receiving Effectto® was lower than that in control; however, this effect was not statistically significant (Table 4, Figure 1, 2).

Table 4. Effect of polyherbal formulation on Body weight in testosterone induced model

Days	Body Weight (g)				
	V.C	D.C	Effectto® (low)	Effectto® (high)	Finasteride
Day 0	210.55±4.49	215.35±5.48	222.15±11.79	220.55±9.83	235.85±7.75
Day 7	220.45±5.48	230.45±8.22	230.50±8.15	230.25±10.49	238.45±9.83
Day 14	235.00±7.75	254.00±10.21	240.11±10.21	245.00±14.49	243.25±10.49
Day 21	250.45±7.53	268.99±5.85	253.11±5.85	258.50±11.29	245.50±9.31
Day 28	260.99±7.75	287.07±10.95	264.50±10.96	270.55±10.80	247.75±8.61

All data are expressed as mean ± SD, n = 6 in each group, Where V.C = Vehicle control; D.C = Disease control.

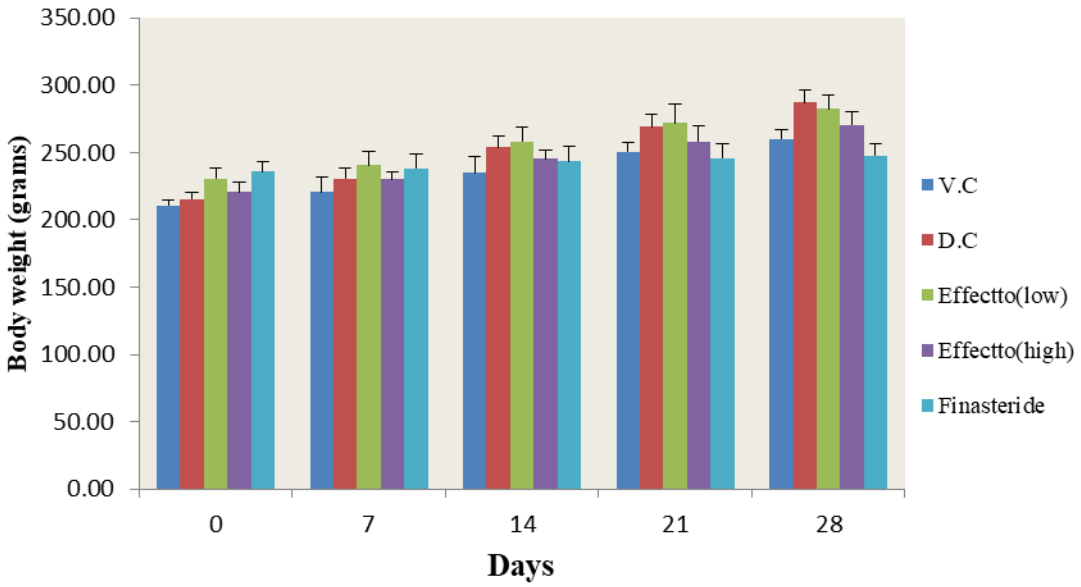


Figure 1. Effect of polyherbal formulation on body weight in testosterone induced BPH model in rats.

All data are expressed as mean ± SD, n = 6 in each group, Where V.C. = Vehicle control; D.C. = Disease control.

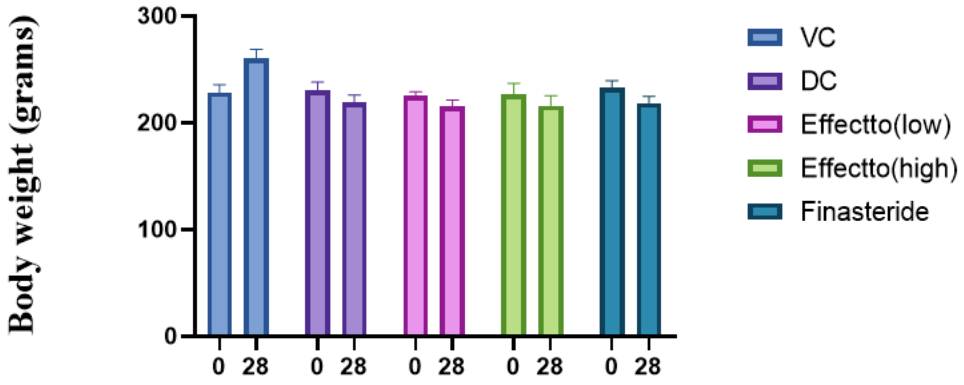


Figure 2. Change in body weight at the end of the study duration.

All data are expressed as mean ± SD, n = 6 in each group, Where V.C. = Vehicle control; D.C. = Disease control

The change in bodyweight in animals receiving testosterone for 28 days and the effect of treatment with Effect to or Finasteride were also studied. As noted earlier, the testosterone-mediated increase in the body weight of the animals was reversed by treatment with both Effect to and Finasteride. However, this effect was statistically significant for Finasteride only ($p<0.05$).

3.3 Prostate Weight Index

At the end of the study period, the rats were euthanised. The ventral prostate was quickly isolated, tissue attachments and debris cleared, and then the prostate was weighed using a digital balance. The prostatic weight index was calculated using the body weight as a base (Figure 3).

3.4 Testis Weight Index

At the end of the study period, the rats were euthanised. The left and right testis were quickly isolated, tissue attachments and debris cleared and then the testis were weighed using a digital balance. The testis weight index was calculated using the body weight as a base.

Chronic exposure to high serum Testosterone is known to cause a reduction in testis weight due to a negative feedback effect at the central level. The same was observed in our study. Testosterone was found to significantly reduce the testis weight index in 28 days. The same effect was reversed by concurrent Finasteride administration but not by Effectto treatment. This suggests that the action of Effectto is possibly more specific on the prostate gland and not so much on the testis (Figure 4).

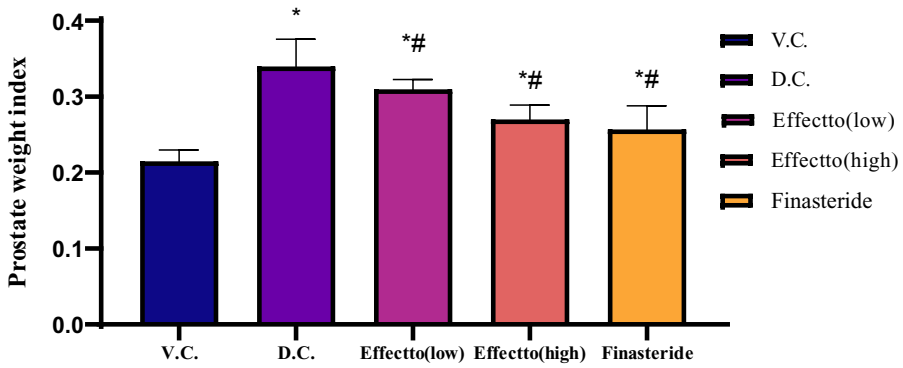


Figure 3. Effect of polyherbal formulation on prostate weight index using testosterone induced BPH model in rats.

All data are expressed as mean ± SD (n = 6 in each group) and analyse one-way ANOVA followed by Tukey's multiple comparison test.
* $p<0.05$ when compared to V.C., # $p<0.05$ when compared to D.C.

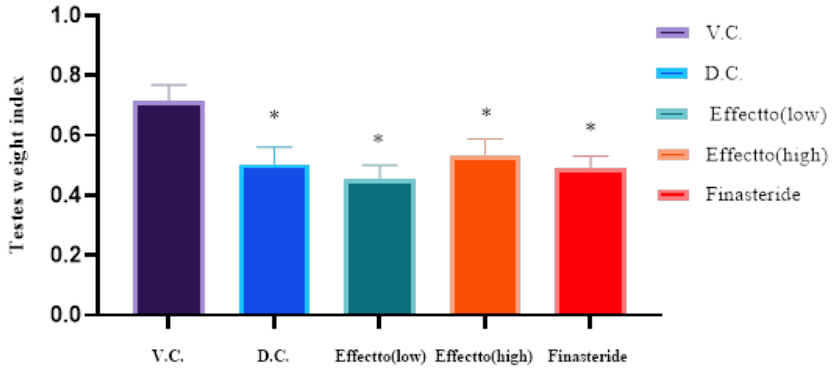


Figure 4. Effect of polyherbal formulation on testis weight index using testosterone induced BPH model.

All data are expressed as mean ± SD, n = 6 in each group, Where V.C = Vehicle control; D.C = Disease control; * p<0.05 when compared to V.C., # p<0.05 when compared to D.C.

3.5 PSA Levels on Testosterone Induced BPH Model

The PSA ELISA kit, SG-20837 was obtained from GenxBio® for determining the levels of serum PSA. PSA was evaluated after terminal sacrifice to study the effect of Effectto® and Finasteride on BPH in a testosterone-induced model in rats. A blood sample for all groups was collected through cardiac puncture at the end of the study. In order to obtain serum, samples were allowed to clot for 10 to 20 min at room temperature and then centrifugation for 20 min at 2000 rpm was done. The supernatant was collected and stored at -20 ºC until further use.

Prostate specific antigen is produced by the prostate gland, is an established marker of prostate gland activity, and is used to diagnose hypertrophy and malignancy of the prostate. The serum PSA was assessed at the end of the study duration to study the effect of the polyherbal formulation Effectto® on the BPH induced by the testosterone induced rat model. Animals receiving testosterone were found to exhibit a significant rise in serum PSA levels when compared with the control group. The PSA was found to be above 4 ng/ml, indicating clinically relevant evidence of prostatic hypertrophy. Treatment with the polyherbal formulation of Effectto® significantly reversed the rise in the levels of serum PSA. Moreover, the effect on serum PSA was dose-dependent. Further, the action of Effectto® at 180 mg/kg was comparable to that of Finasteride (Figure 5).

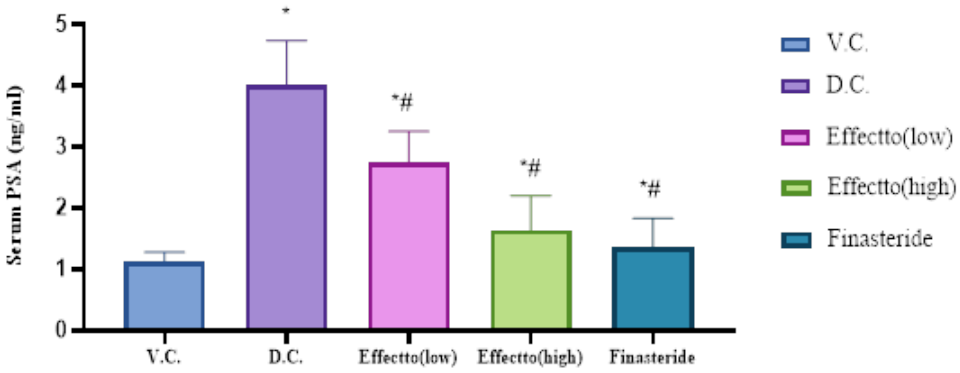


Figure 5. Effect of polyherbal formulation on serum PSA levels in testosterone induced BPH model in rats.

All data are expressed as mean ± SD, n = 6 in each group, *p<0.05 when compared to V.C., # p<0.05 when compared to D.C. Where V.C = Vehicle control, D.C. = Disease control.

3.6 Serum TNF-α

The serum TNF-α level was determined using ELISA kit, ELR TNF-α which was obtained from Ray Biotech. The samples were collected at the end of the study, i.e. after euthanasia. The serum so obtained is stored at -20°C until further use.

From the present study it can be evaluated that the group D.C. showed significant rise in TNF-α levels when compared to V.C. group which explains that the testosteroneincreased the serum TNF-α in the animals as expected from the earlier studies. The Effectto® (high) group showed statistically significant decrease in the levels of TNF- α. The lower dose of the formulation showed a decrease in the levels, but it was found tobe not significant (Figure 6).

3.7 BUN

The BUN levels were assessed to check for any deleterious effects of testosterone and/or Effectto®, or Finasteride on kidney function. Though there was a mild elevation of BUN in the rats exposed to testosterone, it was not found to be statistically significant.Further, the values were also found to be in the clinically normal range (Figure 7).

3.8 Serum Creatinine

The creatinine levels were also assessed to check any harmful effect of testosterone and/or Effectto®, Finasteride on Renal function. Though, there was a mild elevation in the rats exposed to testosterone, it was not found to be statistically significant (Figure 8).

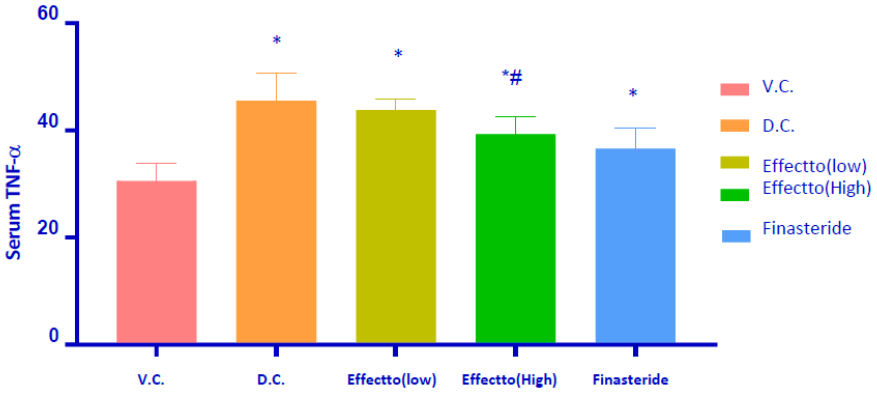


Figure 6. Effect of polyherbal formulation on serum TNF-α level usingtestosterone induced BPH model.

All data are expressed as mean ± SD (n = 6 in each group) and analysedby one- way ANOVA followed by Tukey's multiple comparison test; *p<0.05 when compared to V.C., #p<0.05 when compared to D.C.

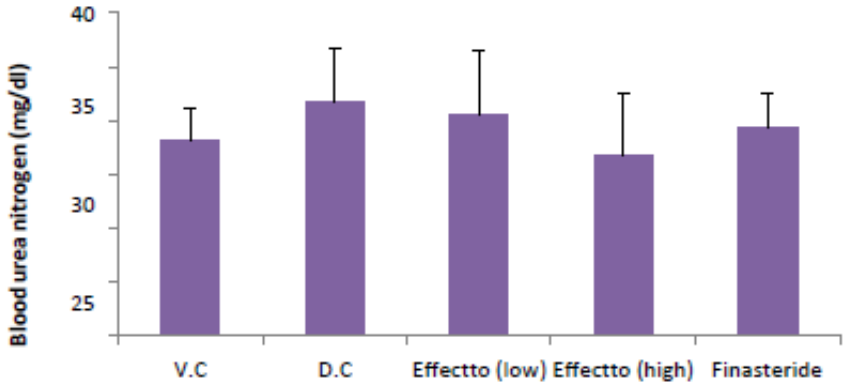


Figure 7. Effect of polyherbal formulation on BUN level in a testosterone-induced BPH model in rats.

All data are expressed as mean ± SD (n = 6 in each group) and analysed by one wayANOVA followed by Tukey's multiple comparison test.

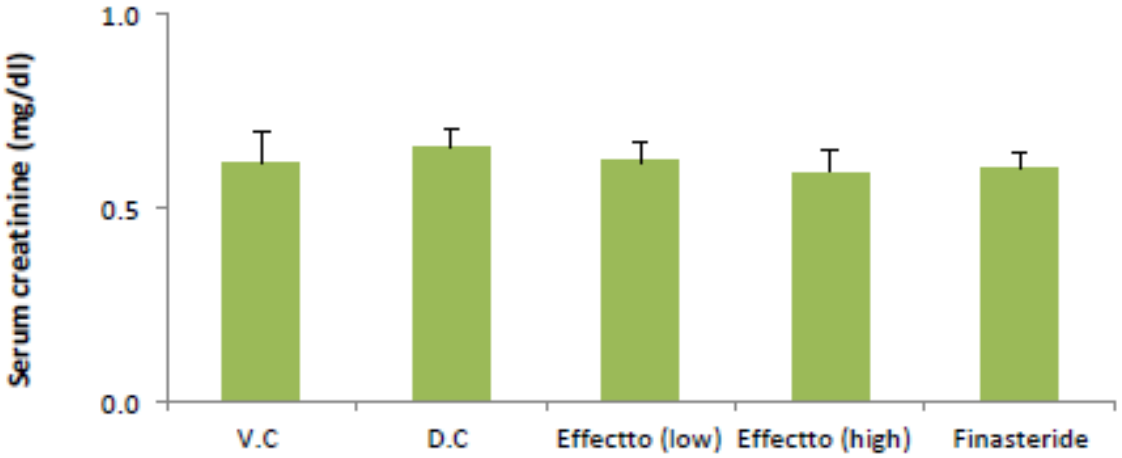
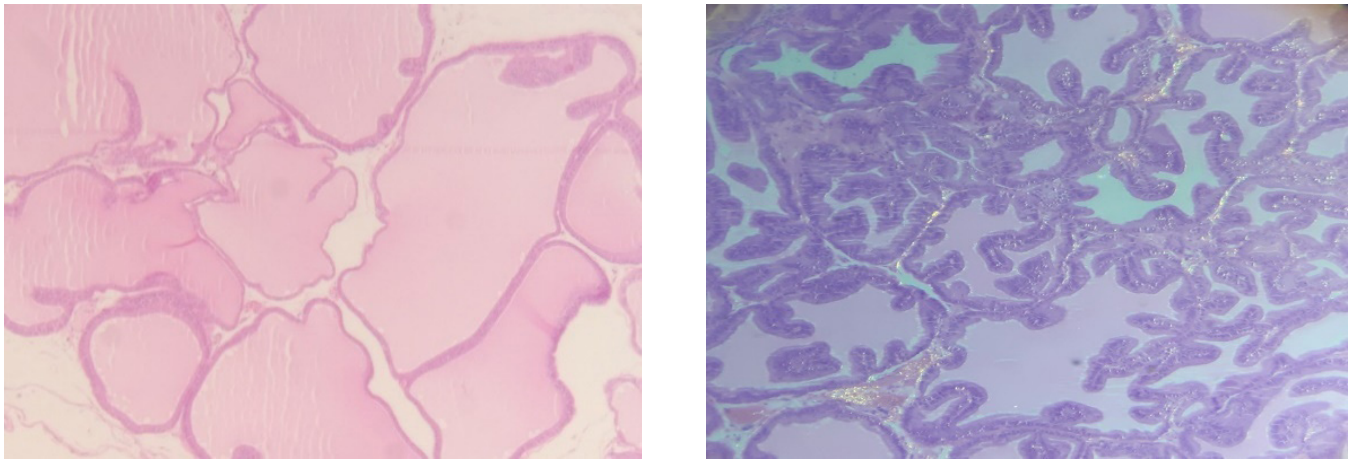


Figure 8. Effect of polyherbal formulation on serum creatinine level intestosterone induced BPH model in rats.

All data are expressed as mean ± SD (n = 6 in each group) and analysed by one-way ANOVA followed by Tukey's multiple comparison test.

3.9 Histopathology

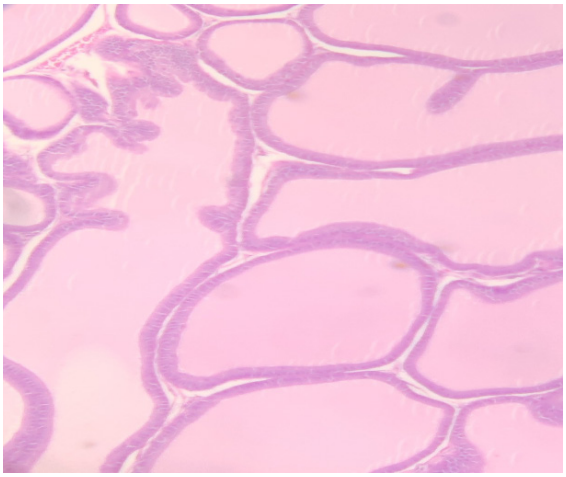
Prostate and testis were collected from the animals after terminal sacrifice. Both prostate and testis were quickly removed, cleaned up, weighed and fixed in 10% neutral buffered formalin solution and stored at 4°C. The samples were then embedded in paraffin and sections (5µm thickness) were cut using a microtome cutter, mounted on a glass slide, stained with H&E stain (hematoxylin-eosin) and observed at 10X and 40X (Figure 9, 10). In the Figure 9, the control group shows much difference in the epithelial lining as well as in the lumen region of the acini as compared to the V.C. The glandular epithelial infolding in the lumen of the D.C. shows a hyperplasic condition. This condition was reversed in both Effectto treatment groups. The Finasteride group as seen in Figure 9, also showed a similar effect.



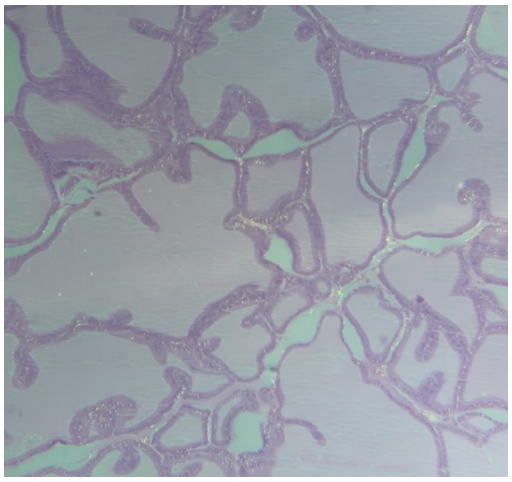
V.C D.C

Figure 9. Histopathological effect of polyherbal formulation on prostate.

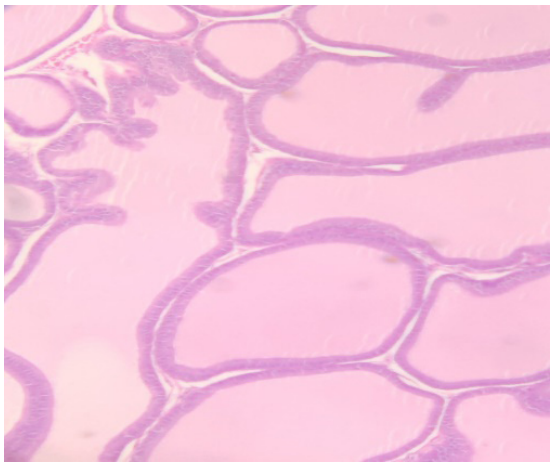
V.C. = Vehicle control, D.C. = Disease control.



Effectto (low)

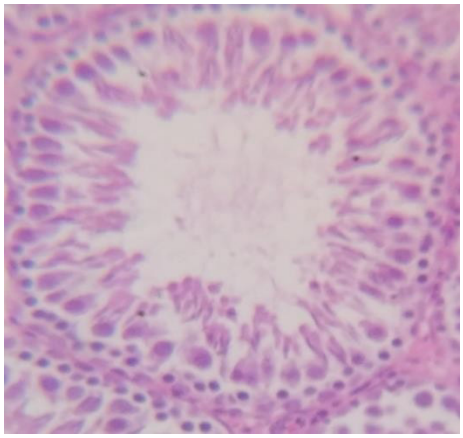


Effectto (high)

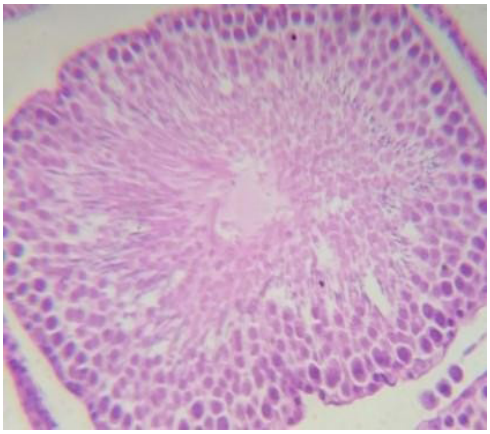


Finasteride

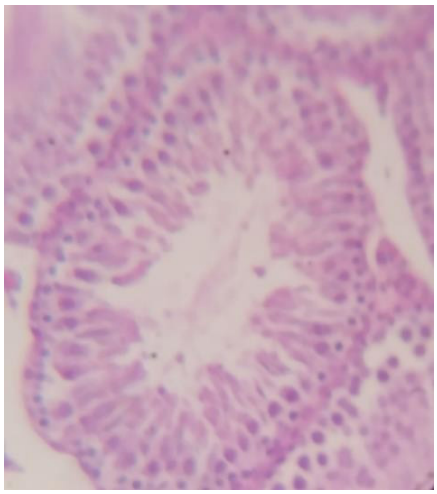
Figure 9. (Continued)



V.C.

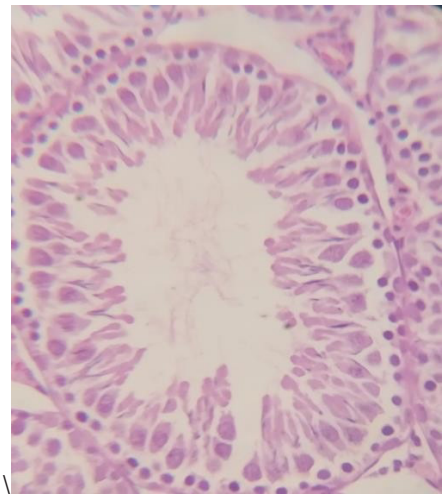


D.C.

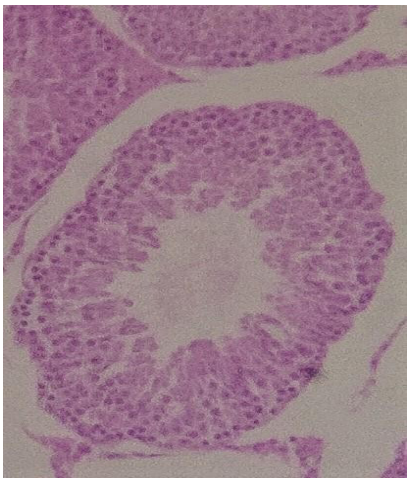


Effectto (low)

Figure 10. Histopathological effect of polyherbal formulation on testis.
V.C. = Vehicle control, D.C. = Disease control



Effectto (high)



Finasteride

Figure 10. (Continued)

In the testosterone-treated group (Figure 10), which was observed under 40X, the vacuole area observed at the centre of the seminiferous tubule is equivalent to null when compared to vehicle control. The histo-architectural changes were observed clearly in the case of disease control, which was not seen in the treatment groups of Effectto as well in Finasteride.

4. Conclusion

Effectto® is a polyherbal formulation designed by Vasu Research Center to be used for the treatment of Benign Prostatic Hyperplasia (BPH). It can also be indicated that it can be used for Lower Urinary Tract Symptoms (LUTS) and for Bladder Outlet Obstruction (BOO). The ingredients that are included in the formulation have been described in traditional literature, or when studied individually, have been found to have anti-inflammatory, anti-proliferative properties, which may contribute to the positive anti- BPH effects of the formulation. The formulation did give a significance relief against testosterone induction. More research is needed to prove that this formulation is effective against atypical type BPH.

Data from the present study demonstrate the successful induction of Benign Prostatic Hyperplasia (BPH) with testosterone. The proprietary polyhedral combination Effectto® was found to cause no morbidity or mortality in rats at a dose of 2000 mg/kg. It also produced a significant reversal in the changes in prostatic tissue and serum PSA levels, as seen in BPH and APH in rats. The action of Effectto® was found to be comparable to that of Finasteride at a dose of 1 mg/kg in some of the parameters. The results from our study provide strong scientific evidence for the use of Effectto® in Benign Prostatic Hyperplasia.

5. Acknowledgement

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