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Acute Oral Toxicity and Efficacy of Uriforte Capsule (Polyherbal Formulation) Against BPH (Benign Prostatic Hyperplasia)

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ABSTRACT

Introduction: Natural therapies have a long history of use in our country to support optimal prostate health. The toxicity profile of newly developed drug is require to provide scientific base and wide acceptance. **Aim:** To evaluate acute oral toxicity of Uriforte capsule (polyherbal formulation) on Swiss albino mice and its efficacy against Benign Prostatic Hyperplasia (BPH). **Method:** The protocol of present study was certified by IAEC (SKPCPER/IAEC/2016-02/03) as per the CPCSEA. The acute oral toxicity was assessed according to OECD guideline AOT-425 to know single dose (2000 mg/kg) toxicity of test drug. The effect of test drug was assessed on Testoviron Depot (TD) injection (2.5mg /kg /day) induced BPH in male Wistar rats. The body weight, urine volume, kidney markers and physical parameters of prostate were analyzed by following provided methods at the end of study. **Results:** There were no physical - behavioral changes and mortality observed in any animal during 14 days. Bodyweight of all animals did not reveal any significant change as compared to vehicle control group. Uriforte capsule showed significant effect on body weight, urine volume and different prostatic and biochemical parameters. All the parameters were normalized in test drug treated group. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Uriforte capsule is 2000 mg/kg as it did not have any toxic effect at that dose. Uriforte capsule might inhibit the 5α-reductase enzyme. The achieved normal value of kidney markers and physical prostate parameter suggests its effectiveness against BPH.

INTRODUCTION

The concept of polyherbal formulation (PHF) is well documented in the ancient literature and they have better and expanded therapeutic potential as compared to the single herb.[1] However, these PHFs are presumed as safe and effective[2] alternative medicines for treatment of various diseases, toxic potential of some herbal combinations is need to be tasted for provide adequate database regarding toxic properties of PHFs.[3]

BPH (Benign Prostate Hyperplasia) is a progressive disease commonly associated with lower urinary tract symptoms (LUTS) such as frequent urination, urgency, nocturia, decreased/intermittent force of stream and sensation of incomplete bladder emptying.[4] Although it is generally not a life threatening condition, it can have a marked effect on a patient's quality of life.[5] The predominant treatment of BPH over the last 60 yrs has been based on various approaches like Watchful waiting[6], drug mono therapy[7], Desmopressin[8], Phyto therapy[9], TURP[10] etc. The aim of therapy for BPH is to improve quality of life by providing symptom relief and increasing maximum flow rate as well as reducing disease progression and development of new morbidities without any side effect.[11] Uriforte capsule is such a PHF indicated for BPH as well as other urinary track problems, burning urination, renal calculi, acute and chronic renal failure.

The present study has been conducted to test the acute oral toxicity of Uriforte capsule to develop its NOAEL and also to establish its efficacy in BPH.

Aim and objectives

To evaluate acute oral toxicity of Uriforte capsule on Swiss albino mice.

To evaluate efficacy of Uriforte capsule against BPH (Benign Prostatic Hyperplasia) induced by Testoviron depot injection in male wistar rats.

MATERIALS AND METHODS

Material: The test drug (Uriforte capsule) was manufactured by following all the GMP standards. The detail of Uriforte capsule is mentioned below;

Table No. 1: Ingredients of Uriforte capsule (Each hard gelatine capsule contain);

Sl. No.	Name of Ingredient	Quantity
1	Ext. *Crataeva nurvala*	100mg
2	Ext. *Boerhavia diffusa*	1000mg
3	Ext. *Tinosporacordi folia*	100mg
4	Ext. *Commiphora weightii*	100mg
5	*Shuddha Shilajita*	100mg

Method: The present study was performed after obtained permission from IAEC (SKPCPER/IAEC/2016-02/03) as per the CPCSEA, Ministry of Environment, Forest and Climate Change (MoFCC), Government of India.

(A) Acute oral toxicity[12]: It was conducted according to OECD guideline AOT-425 to know single dose toxicity of test drug on swiss albino mice. All the animals were acclimatized and kept in proper cages with proper diet. A limit dose of extract (2000 mg/kg) was used in each mouse in sequence at 48 h intervals. The detail of dosing record is as follow;

Table No. 2: Individual animal dosing record

Expt. Day	Animal No.	Gender	Test Drug (mg)	Vehicle Distilled Water (ml)	Volume dosed (ml)	Conc. (mg/ml)
1st day	H	M	50	0.6	0.53	83.33
3rd day	B	M	50	0.6	0.58	83.33
5th day	T	M	55	0.6	0.57	91.67
7th day	HT	M	60	0.6	0.54	100
9th day	UM	M	60	0.6	0.56	100

Expt.: Experiment, **Conc.:** Concentration, **H:** Head, **B:** Body, **T:** Tail, **HT:** Head & Tail, **UM:** Unmarked, **M:** Male, **F:** Female

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a total of 14 days for any clinical signs of toxicity or mortality. Bodyweight of all animals was recorded once in a week.

(B) Effect on BPH: This study was performed in Testoviron depot injection induced BPH in male wistar rats. Animals assigned for study were randomized in four groups (6 animals in each) and maintained in standard condition in accordance with the guideline of the CPCSEA.

Table No. 3: Grouping of Animals

Group No.	Group Name	Dose	No. of animals
I	Vehicle control group (NC)	Olive oil- 1mg/kg/day	6
II	Disease control group (DC)	Injection Testoviron depot 2.5mg/kg/day	6
III	Standard drug treated group (Std.)	Finasteride 1mg/kg/day	6
IV	Uriforte Capsule (UFC)	200mg/kg/day	6

Testoviron depot injection [Testosterone propionate(25 mg/kg) + Testosterone enanthate (250 mg/kg) - Zyduspharma] was given 2.5mg /kg /day through S.C. route in healthy male wistar rats of group II, III and IV for consecutive 21 days to induce BPH. Group III was administered with standard drug [Tab. Finasteride (1mg/kg/day) -Ciplapharma] orally for 21 days. Uriforte Capsule (200mg/kg/day) was given orally in group IV for 21 days.

At the termination of study, Urine volume was measured by keeping them individually in metabolic cages for 6 h. The animals were anesthetized by diethyl ether and blood sample was collected by retro-orbital route for evaluation of kidney markers (parameter analyzer kit - Euro diagnostic systems PVT.LTD.) and Serum dihydrotestosterone (DHT) level (at Supratech Micropath laboratory, Himatnagar). After that rats were euthanized by cervical dislocation and the prostate gland was isolated for the measurement of physical parameters i.e. size, weight, length, width and index.

STATISTICAL ANALYSIS:

Arithmetic mean and standard error of mean are calculated from the individual observations. The data are expressed as mean ± S.E.M. Statistical difference between the mean are calculated using One way analysis of variance (ANOVA) followed by Dunnett's post hoc test. $P<0.05$ is considered statistically significant.

OBSERVATIONS & RESULT

(A) Acute oral toxicity: The animals were observed continuously for behavioural changes, autonomic profiles and other signs of toxicity or mortality up to a period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14th day. There were no physical and behavioural changes observed in Swiss albino mice during 14 days.

Bodyweight of all animals did not reveal any significant change as compared to vehicle control group. Mortality was not observed in any animal of a group.

Table No. 4: Individual animal weekly bodyweight & Mortality record;

Animal No.	Gender	Experiment Day, Unit: gm			Mortality
		1st	7th	14th	
H	M	22	23	24	NIL
B	M	24	25	26	NIL
T	M	26	27	28	NIL
HT	M	27	29	30	NIL
UM	M	28	29	30	NIL

H: Head, **B:** Body, **T:** Tail, **HT:** Head & Tail, **UM:** Unmarked, **M:** Male, **F:** Female

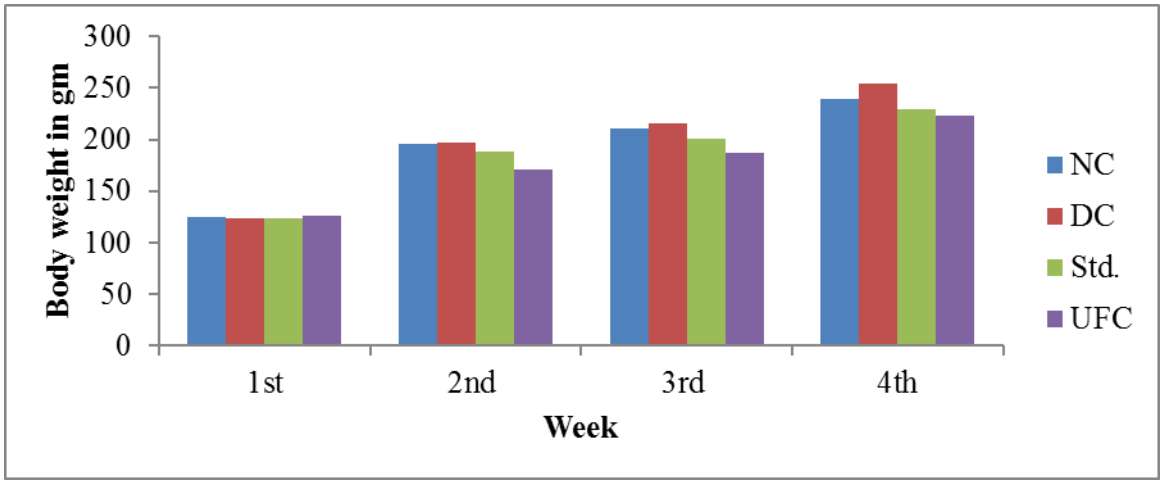
B) Effect on BPH: The effect of test drug on various physical, serum parameters and prostate are as follow;

Urine volume of test drug treated animals was found increased as compared to DC group.

Bodyweight of test drug treated animals was found to be normalized compared to DC group.

Table No. 5: Details of effect on body weight

Group	1st week	2nd week	3rd week	4th week
I (NC)	125	196	211	239
II (DC)	124	197	216	254
III (Std)	124	188	201	230
IV (UFC)	126	171	187	223

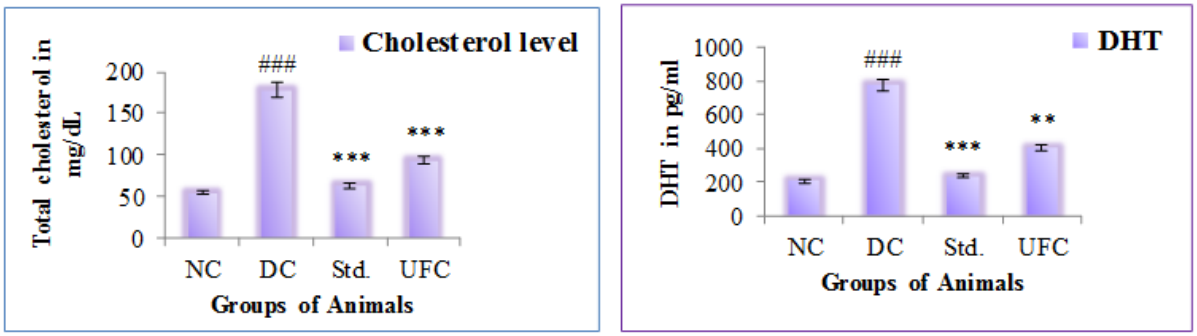


Graph 1: Weekly body weight record of all animals

Table No. 6: Details of effect on serum parameters

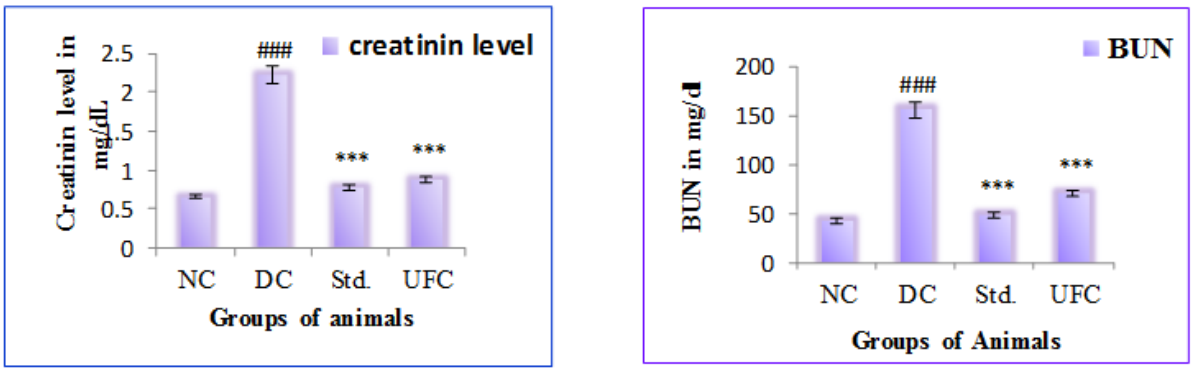
Group	Cholesterol level	Creatinin level	BUN	Total protein	Albumin level	Globulin level	A/G ratio	DHT level
I (NC)	54.67	0.656	42.19	5.36	3.37	1.987	1.81	205
II (DC)	178.3	2.217	156.1	12.94	6.03	6.91	0.98	775
III (Std)	63	0.783	48.09	6.56	3.97	2.597	1.74	235
IV (UFC)	93.33	0.873	70.62	8.65	5.21	3.44	1.543	400

BUN: Blood Urea Nitrogen, DHT: Dihydro- testosterone



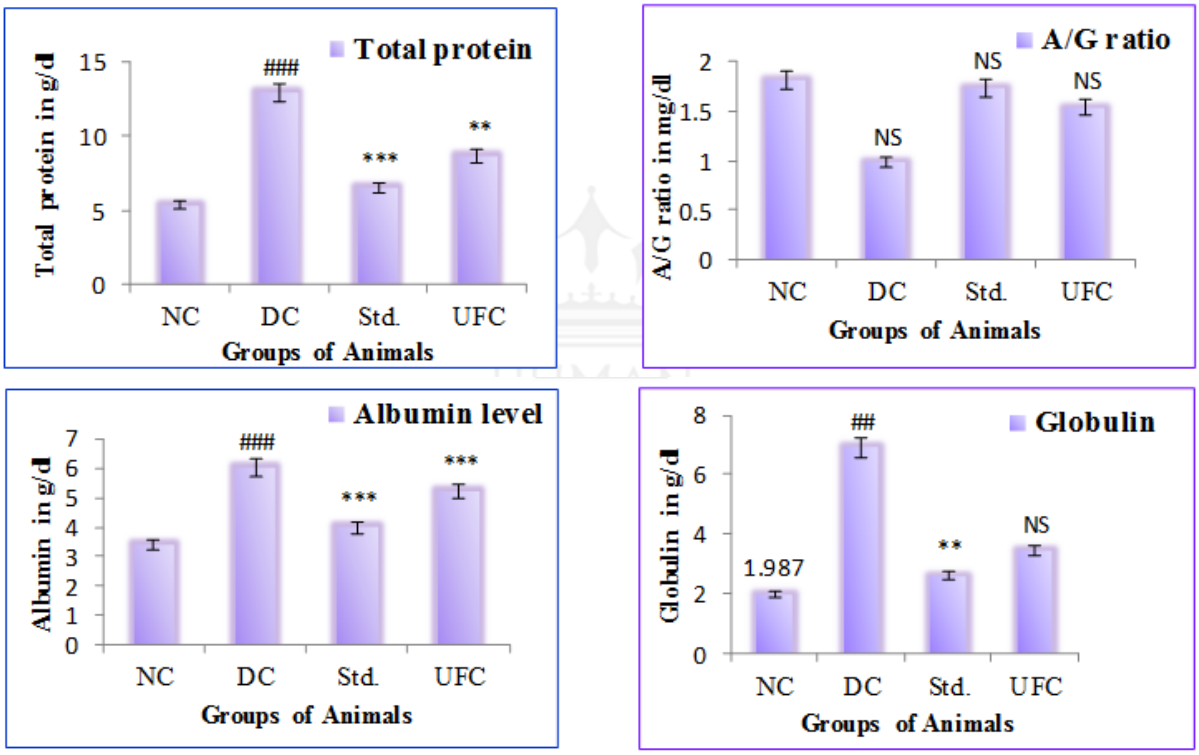
Graph 2: Cholesterol, DHT level (Values are expressed as mean ± S.E.M., n=6)

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control, **P < 0.01 Vs Disease control group



Graph 3: Creatinin, BUN level (Values are expressed as mean ± S.E.M., n=6)

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control



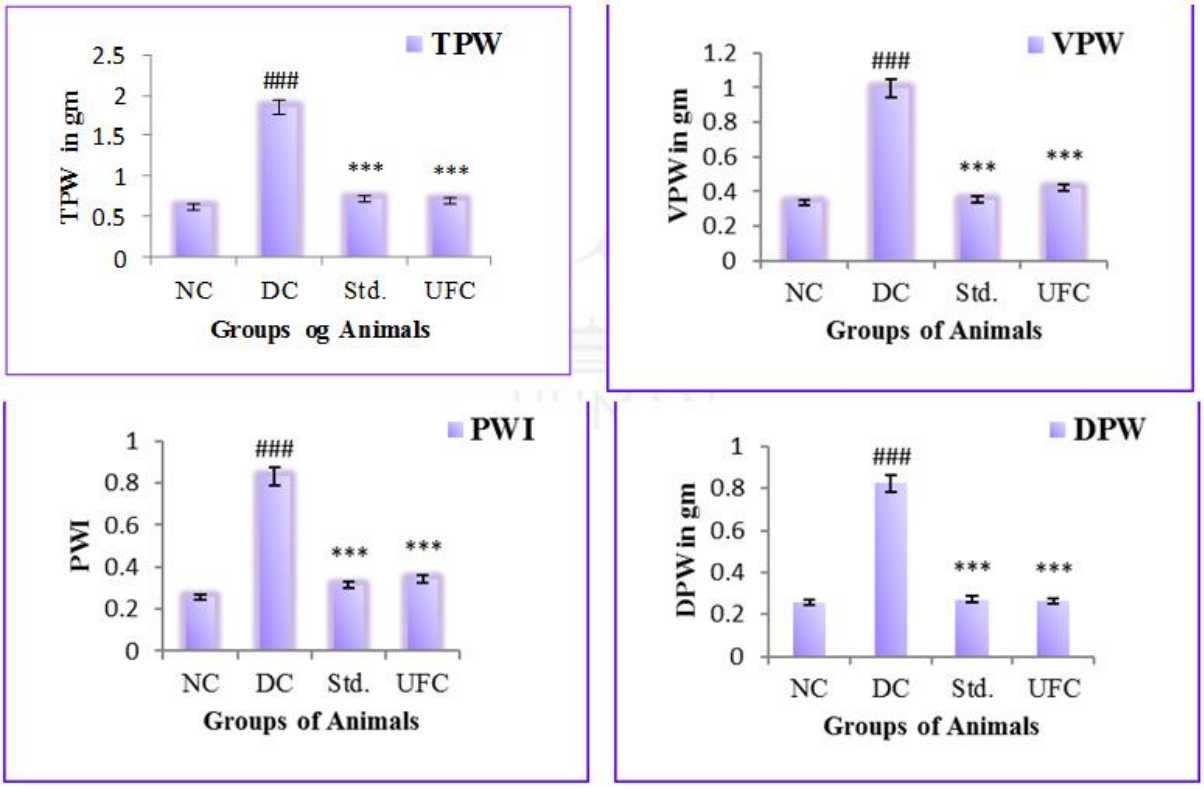
Graph 4: Total protein, Albumin level, Globulin level, A/G ratio (Values are expressed as mean ± S.E.M., n=6)

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control, **P < 0.01 Vs Disease control group, NS: Non significant

Table 7. Details of effect on Prostatic parameters

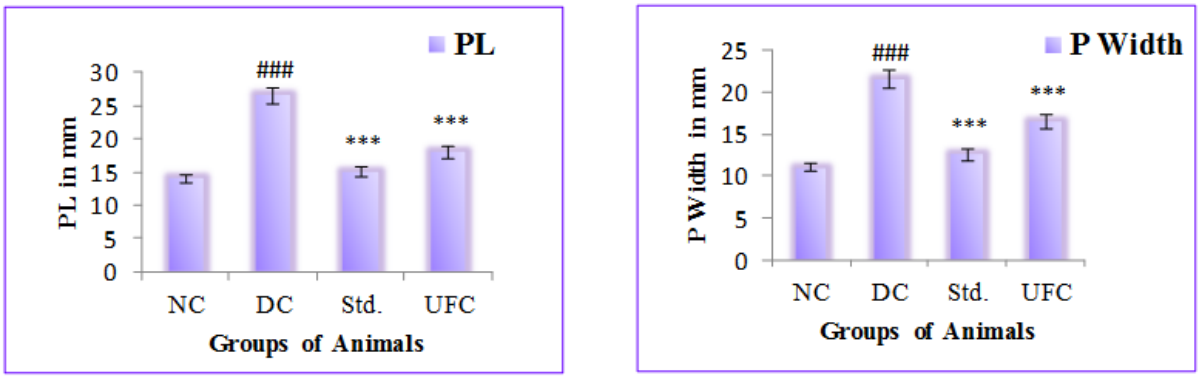
Group	TPW	DPW	VPW	PL	PW	PWI
I (NC)	0.621	0.2564	0.3371	14	11	0.257
II (DC)	1.844	0.8242	0.9966	26.5	21.5	0.832
III (Std)	0.72	0.2721	0.3543	15	12.5	0.312
IV (UFC)	0.695	0.2651	0.4205	18	16.5	0.343

TPW: Total Prostate Weight, DPW: Dorsal Prostate Weight, VPW: Ventral Prostate Weight, PL: Prostate Length, PW: Prostate Width, PWI: Prostate Weight Index



Graph 5: TPW, DPW, VPW, PWI (Values are expressed as mean ± S.E.M., n=6).

$^{###}p < 0.001$ Vs Normal control, $^{***}p < 0.001$ Vs Disease control



Graph 6: PL, PW (Values are expressed as mean ± S.E.M., n=6)

###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control

DISCUSSION

Polyherbal formulations are abundantly used in developed countries as compared to modern medicine for the treatment of various diseases. But sadly their toxicities and side effects are merely known. The herbal toxic effects may be related to intrinsic toxicity, overdosing, herb-drug interaction and contaminated formulations. Therefore toxicity evaluation and screening of bioactive components of herbal medicine should be done.[13] This study can consider as a pioneer step for the establishment of safety profile and efficacy of Uriforte Capsule.

The study was done on Swiss Albino Mice for 14 days to rule out any toxic effect of Uriforte Capsule at the dose of 2000 mg/kg. Individual animal weekly body weight was recorded and found to be increasing during the observation period [**Table 4**]. Animal daily observation was recorded and found to be same and mortality rate was Nil [**Table 4**]. There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Uriforte Capsule which is indicated in BPH has no oral toxicity effect on Swiss albino mice. Hence, this can be used safely for therapeutic purposes.

The Uriforte capsule is a polyherbal formulation containing various potent herbs having proven action on urinary system and prostatic disease. *Crataeva nurvala* has anti inflammatory properties due which it prevents urinary tract infection in BPH [14]. *Borehavia diffusa* has anti inflammatory and anti-proliferative effect which helps in reducing size of prostate [15]. *Shilajita* is a very famous herbo-mineral substance found in the Indian Himalayan region. It is used for treating the inconvenience in urination because of the enlarged prostate gland [16]. The alcoholic extract of *Tinospora cordifolia* has proven anti-

inflammatory actions in models of acute and subacute inflammation [17]. All the ingredient of this formulation has been reported to have different types of anti-BPH effects.

The effect of test drug against BPH was performed in Testoviron depot injection induced BPH in male wistar rats. The bodyweight of test drug treated group was found normal as compared to DC group **[Table 5]**. The urine volume was observed restored and increased in drug treated group as compared to DC group may be due to its diuretic and anti inflammatory properties. UFC brought DHT level nearby normal **[Table 6]** which indicates its 5α-reductase enzyme inhibitory property. The significant decrease was found in kidney markers (cholesterol, BUN, creatinine, total protein, albumin, globulin, A/G ratio) [**Table 6**] and Prostatic parameters (weight, size, length, width, index) [**Table 7**] in test drug treated group as compared to DC group which proves its potential effect in BPH.

CONCLUSION:

The No-Observed-Adverse-Effect-Level (NOAEL) of Uriforte Capsule is 2000 mg/kg as it did not have any toxic effect at that dose. Uriforte capsule might inhibit the 5α-reductase enzyme and has diuretic & anti inflammatory properties. The found normalized value of kidney markers and prostatic parameters suggest its effectiveness against BPH.

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