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ACUTE ORAL TOXICITY AND EFFICACY OF URINEJ CAPSULE (POLY-HERBAL 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 requiring providing scientific base and wide acceptance. **Aim:** To evaluate acute oral toxicity of Urinej capsule (poly herbal 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. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Urinej 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) Urinej capsule is 2000 mg/kg as it did not have any toxic effect at that dose. Urinej capsule might inhibit the 5α-reductase enzyme. The achieved normal value of kidney markers and physical prostate parameter suggests its effectiveness against BPH.

KEYWORDS: Poly herbal formulation, Urinej capsule, NOAEL, Mortality, OECD Guideline.

INTRODUCTION

The concept of poly herbal 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] Urinej 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 Urinej capsule to develop its NOEL and also to establish its efficacy in BPH.

AIM AND OBJECTIVES

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

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

MATERIALS AND METHODS

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

Table 1: Ingredients of Urinej capsule (Each hard gelatine capsule contain).

Sl. No.	Name of Ingredient	Quantity
1	Ext. *Crataeva nurvala*	125mg
2	Ext. *Boerhavia diffusa*	125mg
3	Ext.*Shuddha Shilajita*	125mg
4	Ext. *Commiphora wightii*	125mg

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 02: 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	60	0.6	0.58	100
5th day	T	M	60	0.6	0.56	100
7th day	HT	M	60	0.6	0.58	100
9th day	UM	M	55	0.6	0.56	91.67

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. Body weight 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 03: 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	Urinej Capsule (UC)	200mg/kg/day	6

Testoviron depot injection [Testosterone propionate(25 mg/kg) + Testosterone enanthate (250 mg/kg) - Zydus pharma] 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) - Cipla pharma] orally for 21 days. Urinej 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 dihydro-testosterone (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 AND 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. Body weight 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 04: Individual animal weekly body weight & Mortality record.

Animal No.	Gender	Experiment Day, Unit : gm			Mortality
		1st	7th	14th	
H	M	22	23	24	NIL
B	M	29	30	31	NIL
T	M	28	29	30	NIL
HT	M	29	30	31	NIL
UM	M	26	27	28	NIL

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

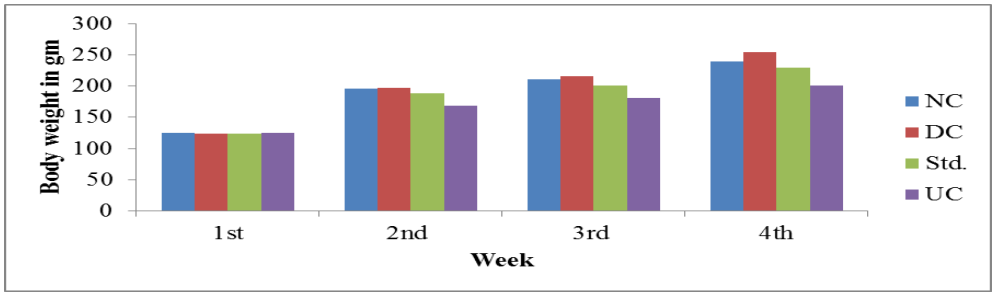
Table 05: Details of effect on body weight.

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 (UC)	125	169	181	201

(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.

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

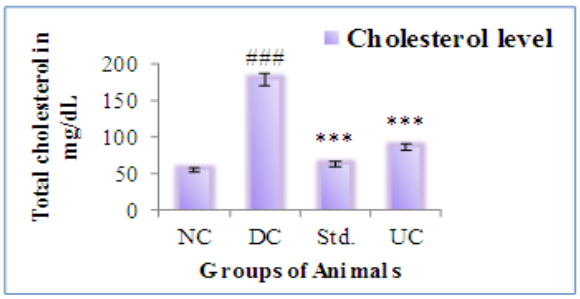


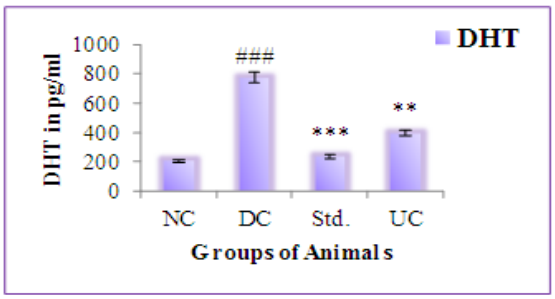
Graph 01: Weekly body weight record of all animals.

Table 06: 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 (UC)	86	0.846	69.44	9.16	4.87	4.297	1.143	397.5

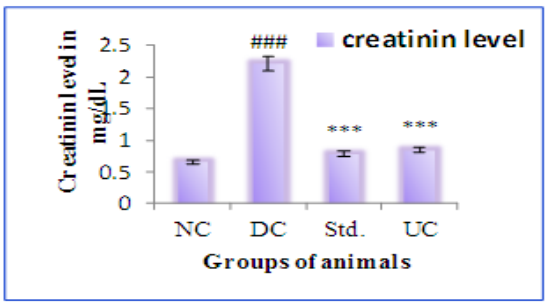
BUN: Blood Urea Nitrogen, DHT: Dihydro-testosterone

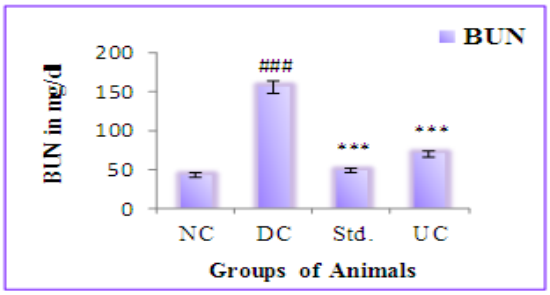




###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control, **P < 0.01 Vs Disease control group

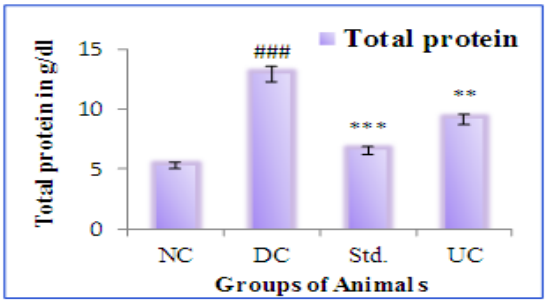
Graph 02: Cholesterol, DHT level (Values are expressed as mean ± S.E.M., n=6).

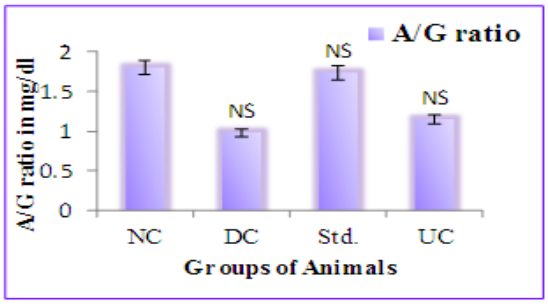


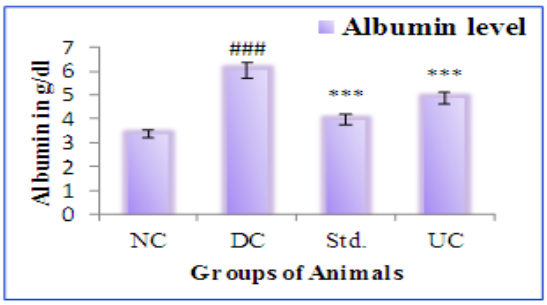


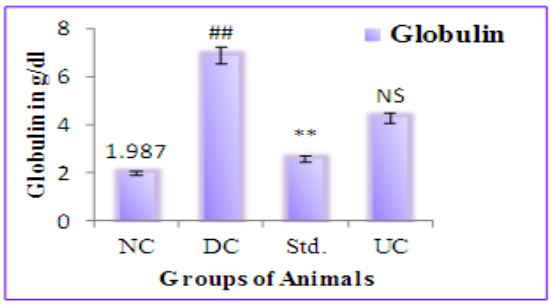
###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control

Graph 03: Creatinin, BUN level (Values are expressed as mean ± S.E.M., n=6).









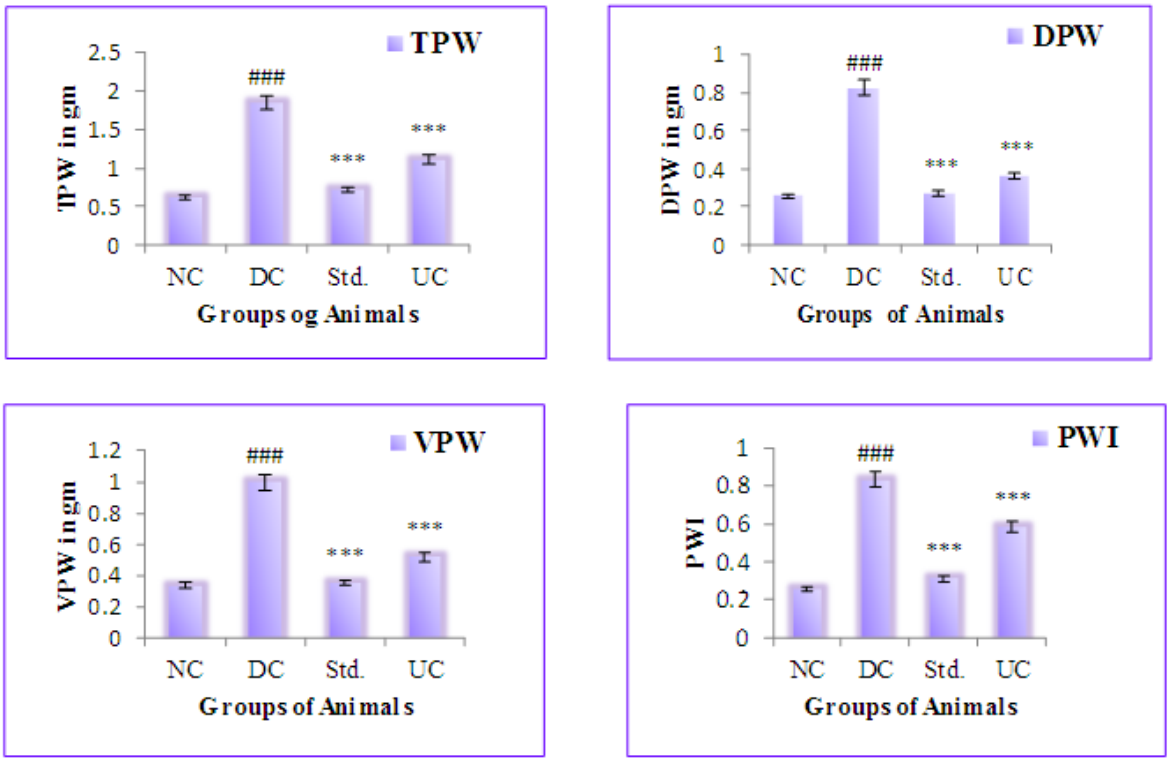
###p < 0.001 Vs Normal control, ***p < 0.001 Vs Disease control, **P < 0.01 Vs Disease control group, NS: Non significant

Graph 04: Total protein, Albumin level, Globulin level, A/G ratio (Values are expressed as mean ± S.E.M., n=6).

Table 07: Details of effect on Prostatic parameters.

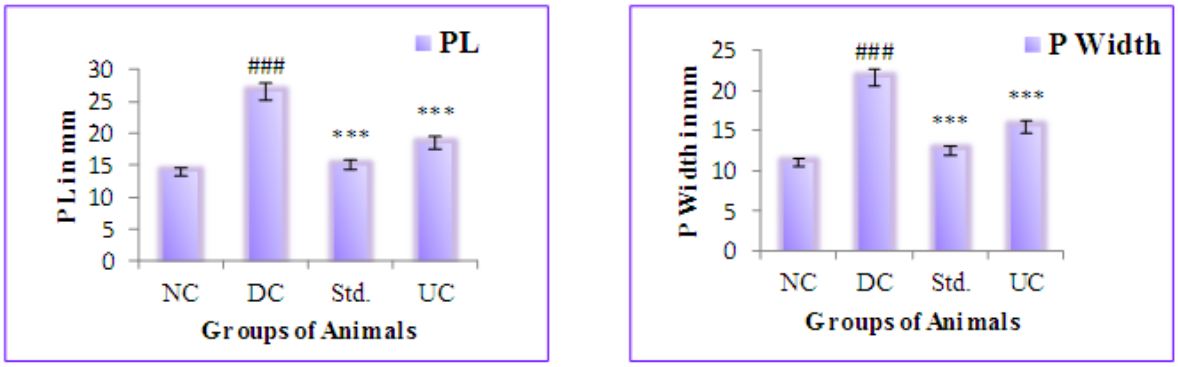
Group	TPW	DPW	VPW	PL	PW	PWI
I (NC)	0.621	0.2564	0.3371	14	11	0.257
II (DC)	1.844	0.8242	0.9966	26.5	21.5	0.832
III (Std)	0.72	0.2721	0.3543	15	12.5	0.312
IV (UC)	1.114	0.3636	0.5183	18.5	15.5	0.5815

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



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

Graph 05: 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).

DISCUSSION

Poly herbal 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, over dosing, 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 Urinej Capsule.

The study was done on Swiss Albino Mice for 14 days to rule out any toxic effect of Urinej 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 04). Animal daily observation was recorded and found to be same and mortality rate was Nil (Table 04). There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Urinej 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 Urinej capsule is a ploy-herbal 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 poliperative 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] *Commiphora wightii* is herbal drug has anti-inflammatory, diuretic, use in turbidity of urine and urinary calculus. All drugs of formulations are well reported in Ayurvedic texts and scientific research publications for anti-inflammatory, diuretic, antioxidant and 5αreductase activity. 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 body weight of test drug treated group was found normal as compared to DC group (Table05). 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 o6) which indicates its 5α-reductase enzyme inhibitory property. The significant decrease was found in kidney markers (cholesterol, BUN, creatinin, total protein, albumin, globulin, A/G ratio) (Table o6) and Prostatic parameters (weight, size, length, width, index) (Table o7) 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 Urinej Capsule is 2000 mg/kg as it did not have any toxic effect at that dose. Urinej 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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1. Petalad Mahal Arogya Mandal Pharmacy (PMAMP), At. Po. Pipalata - , Dist. Kheda, Gujarat, India.
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