CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3403516

Available online at: http://www.iajps.com *Research Article*

COMBINATORIAL EFFECTS OF METHANOL EXTRACT OF IMPERATA CYLINDRICA AND JATHROPHA CURCAS ROOTS ON TESTOSTERONE-INDUCED BENIGN PROSTATE HYPERPLASIA (BPH) IN MALE ALBINO RATS

[1]Ganyam, M. M, [2]Arazu A. V, [3,4*]Amadi Ben C, [5]Itepu Victor E, [6]Ugwuoke K.C, [7]Samuel C.

[1]Department of Biochemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria, [2]Department of Science Laboratory Technology University of Nigeria, Nsukka, Nigeria, [3]Institute for Drug-Herbal Medicine-Excipient Research and Development UNN, [4]Department of Pharmaceutical Technology, University of Nigeria, Nsukka, Nigeria, [5]Department of Biochemistry, Edo University Iyamho, Edo State. Nigeria [6]Department of Biochemistry Federal University Wukari. Taraba State, Nigeria, [7]Department of Biochemistry University of Nigeria, Nsukka.

Article Received: July 2019 **Accepted:** August 2019 **Published:** September 2019

Abstract:

This study was aimed at evaluating the effects of methanol extract of Imperata cylindrica and Jathropha curcas roots on testosterone-induced benign prostate hyperplasia (BPH) in male albino rats. A total of 27 male albino rats were divided into 9 Groups of 3 rats each. BPH was induced using 25 mg/kg b.w of testosterone propionate subcutaneously for 28 days in all the groups with exception of the normal control group, followed by treatment immediately, for 14 days. Biochemical assays such as liver function test (ALT, AST and ALP), kidney function test (Urea and Creatinine), antioxidant (SOD and CAT), MDA, testosterone, zinc concentration and prostate specific antigen (PSA) levels were analyzed using standard procedures. Prostate weight (prostate/body weight ratio) and percentage inhibition of prostate weights, were also calculated. The result obtained revealed a decrease in mean body weight from day 1 to day 14. The results showed a non-significant ($p > 0.05$) difference in the level of liver enzymes. The Malondialdehyde (MDA) concentrations significantly ($p < 0.05$) decreased was observed within the groups. A noticeable change was also observed in antioxidants enzymes. The prostate weight significantly decreased when decrease in prostatic index. These results suggest that methanol extract of Jathropha curcas roots and its combination with Imperata cylindrical may be effectively used in the management of BPH.

Key words: *Imperata cylindrical roots, Jathropha curcas roots, testosterone and benign prostate hyperplasia (BPH)*

Corresponding author:
Amadi Ben C,
Department of Pharmaceutical Technology,
University of Nigeria, Nsukka, Nigeria
ben.amadi@unn.edu.ng

QR code

Please cite this article in press Amadi Ben C et al., ***Combinatorial Effects Of Methanol Extract Of Imperata Cylindrica And Jathropha Curcas Roots On Testosterone-Induced Benign Prostate Hyperplasia (Bph) In Male Albino Rats.,*** *Indo Am. J. P. Sci, 2019; 06(09).*

INTRODUCTION:

Benign prostatic hyperplasia (BPH) is among the most common urological abnormality affecting the aging male of above 40 years age. The cause of the increase in prostatic volume is numerous, but current research has implicated hormonal abnormality. Benign prostate hyperplasia (BPH) disease is characterized by uncontrolled proliferation of the prostate epithelial cells and stromal cells, which results in increased prostate size [1]. As prostate enlarges, it constricts the urethra and reduces urine outflow, thus creating lower urinary tract symptoms (LUTS). Clinical BPH is commonly viewed as benign enlargement of the prostate, which contributes to an array of urinary voiding difficulties among older men [2]. The hypertrophy of prostate, caused by excessive dihydrotestosterone (DHT) is estimated as the mechanism that oversupplies testosterone in blood and leads to large amount of DHT synthesis through the action of 5α-reductase in the prostate. The synthesized DHT combines with androgen receptor with consequent generation of benign prostate hyperplasia [3].

The prevalence of pathological BPH is 8% in the 4th decade of life; however, 50% of men develop pathological BPH between 51 and 60 years. The average weight of a prostate identified at autopsy as having BPH is 33 ± 16 g. Only 4% of the prostates in men older than 70 years weigh >100 g. Globally, benign prostatic hyperplasia affected about 210 million males in 2010 (6% of the population) [4]. Conventional drug treatment includes 5 alpha reductase inhibitors and alpha-adrenergic antagonists. Although these drugs have great efficacy in treating patients, their adverse effects like impotence, gynecomastia, impairment of muscle growth and decreased libido for 5 alpha reductase inhibitors and orthostatic hypotension, fatigue, dizziness, abnormal ejaculation for alpha adrenergic antagonists should not be over looked. People in the developing countries have resorted to depending on herbal medicines for their health care needs due to, the adverse effects observed with the conventional drugs, long term surgical treatments which are costly as well as the risk for aged men [5].

Capsicum annuum L. (Bell pepper) is grown in almost every area in the world. It is the second-most consumed vegetable worldwide and is characterized by its high levels of vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium [6]. Mature pepper fruits are also rich in carotenoids, compounds with antioxidant and anti-carcinogenic capacity; furthermore, either immature or mature fruits contain a high concentration of antioxidant phenolic compounds [7]. *Imperata cylindrica* and Capsicum species roots extract have been claimed by traditionalist to have remedial effects on BPH. The presence of these phytochemicals with different bioactivity in the plants, suggest their therapeutic potentials for the management of BPH.

MATERIALS AND METHODS:

Induction of benign prostate hyperplasia (BPH):

Benign prostate hyperplasia (BPH) was induced by subcutaneous injection with 25 mg/kg b.w of Sustanon^{R} '250' (Testosterone esters), manufactured by Pharmatec Pakistan LTD under license from N.V. Organon OSS, The Netherlands for 28 days in male rats to be used for this study with exception of Group 1 (Normal control) [8].

Experimental design:

A total of twenty seven (27) albino male rats were used. They were acclimatized to laboratory conditions for a period of one week and all rats had access to pelletized feed (Chikum feeds) and water ad libitum. They were randomly distributed into nine (9) groups of three (3) animals each. The study lasted for 14 days.

The experimental groups were as follows:

Group 1: Normal Control
Group 2: Positive control
Group 3 (Standard control): Received Dutasteride 0.5 mg/kg b.w
Group 4: Received 50 mg/kg body weight of methanol extract of *Imperata cylindrica* roots
Group 5: Received 100 mg/kg body weight of methanol extract of *Imperata cylindrica* roots
Group 6: Received 50 mg/kg body weight of methanol extract of ripe *capsicum annum* fruits
Group 7: Received 100 mg/kg body weight of methanol extract of ripe *capsicum annum* fruits
Group 8: Received methanol extract of *Imperata cylindrica* roots and ripe *capsicum annum* fruits (50 mg/kg b.w)
Group 9: Received methanol extract of *Imperata cylindrica* roots and ripe *capsicum annum* fruits (100 mg/kg b.w)

Plant extraction method:

Imperata cylindrical roots and ripe *capsicum annum* fruits were washed, dried and then ground into powder using milling machine. The powdered roots (100 g each) and fruits were soaked in 3 litres of 70% methanol for 48 hours separately. The extracts were filtered first with a muslin clothe and further filtered

using whatsman filter paper. The filtrate obtained was concentrated in a rotary evaporator at 60^0C, and then dried with regulated water bath at 20^0 C given a light brown yield of 12.5 g each.

Biochemical Assays:
Liver function test was carried out by the methods as stated in RANDOX Commercial Enzyme kit (RCA) test kit.

Prostate weight to body weight ratio (Prostate index):
Prostate weight to body weight ratio were calculated by dividing prostate weight with that of animal body weight multiplied by 100 for the individual study group animal.

Percentage increase in prostate weight:
The percentage increase was calculated by dividing the prostate weight of the individual test groups with that of the positive control and multiplied by 100.
Percentage inhibition of increase in prostate weight
Percentage of inhibition was calculated as follows:
100 – {[(PW of treated group-PW of negative control)/(PW of positive group-PW of negative control)]×100}
Where PW: Prostate Weight [9].

Statistical Analysis:
The results were expressed as mean ± standard error of mean (SEM) and test of statistical significance was carried out using one-way analysis of variance (ANOVA).The data obtained were analyzed using IBM Statistical Products and Service Solutions (SPSS), Version 16. Values with $p < 0.05$ were considered statistically significant.

RESULTS:

Table 1: Quantitative phytochemical constitutients of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots

Phytochemicals	*Imperata cylindrica* roots extract (mg/g)	*Jathropha curcas* roots extract (mg/g)
Tannins	5.80 ± 0.16	7.49 ± 0 .50
Hydrogen cyanide	1.66 ± 0.07	5.32 ± 0.14
Flavonoids	2.53 ±0 .22	11.21 ± 0 .35
Reducing sugars	18.41 ± 0.51	19.78 ± 2.18
Phenols	12.88 ± 0 .27	13.05 ± 0 .40
Alkaloids	4.65 ± 0.13	3.53 ± 0.15
Steroids	0.54 ± 0.03	0.48 ± 0.04
Terpenoids	13.20 ± 0 .20	15.10 ± 0 .45
Soluble carbohydrates	2.40 ± 0 .05	2.45 ± 0.14

Results are expressed as mean ± SEM; n=3

Table 2: Effects of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots on body weight of BPH induced male albino rats

Groups	Animal Weight (g)		% Weight gain/loss
	Day 1	Day 14	
1	134.47 ± 6.02[a]	154.77 ± 6.40[a]	13.12
2	142.37 ± 12.52[a]	129.84 ± 19.41[a]	8.80
3	153.00 ± 9.62[a]	126.36 ± 14.35[a]	17.41
4	139.13 ± 10.83[a]	129.01 ± 10.94[a]	7.27
5	154.77 ± 5.47[a]	143.05 ± 6.11[a]	7.57
6	149.03 ± 15.24[a]	129.01 ± 10.93[a]	13.43
7	148.06 ± 10.42[a]	143.04 ± 6.10[a]	3.37
8	168.86 ± 15.61[a]	158.19 ± 13.11[a]	6.31
9	157.33 ± 5.53[a]	127.93 ± 19.07[a]	18.68

Values are expressed as mean ± SEM ;(n =3).Values with the same superscript are considered not statistically significant at $p > 0.05$

Table 3: Effects of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots on some liver and kidney parameters in serum of BPH induced male albino rats

Groups	AST(iu/L)	ALT (iu/L)	ALP(iu/L)	T.BIL (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
1	40.33 ± 3.18^{ab}	76.33 ± 3.18^{bc}	15.63 ± 1.23^{c}	0.72 ± 0.30^{b}	19.77 ± 0.43^{a}	0.68 ± 0.02^{a}
2	34.33 ± 0.88^{a}	60.00 ± 1.15^{ab}	13.30 ± 0.17^{b}	0.66 ± 0.11^{ab}	19.43 ± 1.68^{a}	0.64 ± 0.03^{a}
3	35.33 ± 4.91^{a}	60.66 ± 4.37^{ab}	12.33 ± 0.72^{ab}	0.44 ± 0.05^{ab}	24.43 ± 4.52^{a}	0.88 ± 0.13^{a}
4	37.00 ± 8.15^{a}	74.00 ± 2.65^{c}	11.10 ± 0.40^{ab}	0.34 ± 0.06^{ab}	20.07 ± 0.72^{a}	0.67 ± 0.02^{a}
5	28.33 ± 2.60^{a}	88.00 ± 8.54^{bc}	11.40 ± 0.40^{ab}	0.47 ± 0.05^{ab}	22.53 ± 0.62^{a}	0.69 ± 0.02^{a}
6	38.33 ± 3.18^{a}	54.00 ±7.81^{a}	10.63 ± 0.45^{a}	0.31 ± 0.02^{a}	20.93 ± 1.02^{a}	0.66 ± 0.01^{a}
7	45.00 ± 3.46^{ab}	59.00 ± 0.58^{ab}	12.30 ± 1.10^{ab}	0.32 ± 0.01^{a}	22.02 ± 0.90^{a}	0.76 ± 0.07^{a}
8	54.00 ± 9.64^{b}	51.00 ± 7.21^{a}	13.40 ± 0.72^{b}	0.52 ± 0.11^{ab}	20.90 ± 0.72^{a}	0.82 ± 0.06^{a}
9	43.33 ± 4.26^{ab}	50.33 ± 5.55^{a}	10.53 ± 0.32^{a}	0.32 ± 0.02^{a}	23.30 ± 1.79^{a}	0.96 ± 0.27^{a}

Values are expressed as mean ± SEM ;(n =3).Values with different letters as superscript are considered statistically significant at $p < 0.05$

Table 4: Effects of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots on MDA and some antioxidant parameters in benign prostate hyperplasia (BPH) induced male albino rats.

Groups	MDA (mg/ml)	SOD (iu/l)	CAT (iu/l)
1	0.53 ± 0.23^{a}	9.62 ± 0.58^{ab}	8.14 ± 2.92^{abc}
2	1.85 ± 0.12^{ab}	10.15 ± 0.06^{ab}	2.95 ± 1.04^{a}
3	2.10 ± 1.21^{ab}	13.13 ± 1.78^{bc}	11.55 ± 1.33^{cd}
4	3.58 ± 0.33^{bc}	10.03 ± 0.01^{ab}	7.74 ± 0.73^{abc}
5	4.93 ± 0.23^{c}	11.03 ± 0.05^{ab}	9.57 ± 1.47^{bcd}
6	2.30 ± 0.54^{ab}	8.69 ± 0.67^{a}	5.10 ± 2.48^{ab}
7	1.28 ± 0.39^{ab}	10.11 ± 0.08^{ab}	12.81 ± 1.21^{cd}
8	2.33 ± 1.14^{ab}	12.48 ± 1.78^{ab}	10.51 ± 2.56^{bcd}
9	0.42 ± 0.06^{a}	16.06 ± 0.23^{c}	15.16 ± 0.75^{d}

Values are expressed as mean ± SEM ;(n =3).Values with different superscript are considered statistically significant at $p < 0.05$

Table 5: Effects of methanol extracts of Imperata *cylindrica* and Jathropha *curcas* roots on testosterone and zinc in serum of benign prostate hyperplasia (BPH) induced albino male rats

Groups	Testosterone (ng/ml)	Zinc (mg/dl)
1	1.75 ± 0.03^{ab}	192.15 ± 15.10^{d}
2	2.00 ± 0.09^{b}	162.83 ± 4.67^{b}
3	1.90 ± 0.05^{b}	166.30 ± 6.50^{bc}
4	1.73 ± 0.04^{ab}	157.50 ± 2.77^{ab}
5	1.67 ± 0.08^{ab}	174.73 ± 70.16^{bcd}
6	1.62 ± 0.10^{ab}	136.46 ± 6.55^{a}
7	1.57 ± 0.26^{ab}	188.50 ± 14.29^{cd}
8	1.83 ± 0.03^{b}	179.40 ± 27.02^{bcd}
9	1.34 ± 0.19^{a}	191.13 ± 14.45^{cd}

Values are expressed as mean ± SEM ; (n =3).Values with different superscript are considered statistically significant at $p < 0.05$

Table 6: The effects of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots on Prostate Specific Antigen (PSA) in benign prostate hyperplasia induced of male albino rats

Groups	PSA(ng/ml) Day 1(No Treatment)	PSA (ng/ml) Day 14 (Treatment)
1	0.81 ± 0.04	1.01 ± 0.07^{bc}
2	1.14 ± 0.01	1.25 ± 0.01^{d}
3	1.18 ± 0.05	1.09 ± 0.12^{cd}
4	1.35 ± 0.21	0.92 ± 0.04^{abc}
5	1.39 ± 0.24	0.81 ± 0.07^{a}
6	1.33 ± 0.17	0.84 ± 0.03^{ab}
7	1.21 ± 0.07	0.85 ± 0.04^{ab}
8	1.22 ± 0.06	1.03 ± 0.05^{bc}
9	1.15 ± 0.03	1.00 ± 0.03^{abc}

Values are expressed as mean ± SEM ; (n =3).Values with different superscript are considered statistically significant at $p < 0.05$

Table 7: Effects of methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots on prostate weight parameters in benign prostate hyperplasia (BPH) induced male albino rats..

Groups	PW(g)	PW Index (g)	% Increase in PW	% Inhibition of PW
1	0.40 ± 0.14^{a}	0.25 ± 0.09^{a}	-	-
2	0.96 ± 0.052^{c}	0.72 ± 0.05^{c}	-	-
3	0.77 ± 0.047^{bc}	0.61 ± 0.05^{bc}	80.0	33.93
4	0.71 ± 0.01^{b}	0.51 ± 0.08^{bc}	68.4	42.9
5	0.65 ± 0.06^{b}	0.49 ± 0.02^{bc}	74.0	53.6
6	0.83 ± 0.03^{bc}	0.63 ± 0.07^{bc}	87.3	21.43
7	0.60 ± 0.12^{ab}	0.49 ± 0.12^{b}	63.1	60.5
8	0.68 ± 0.01^{b}	0.45 ± 0.04^{ab}	74.7	42.8
9	0.60 ± 0.03^{ab}	0.49 ± 0.09^{bc}	63.1	62.5

Values are expressed as mean ± SEM ;(n =3).Values with different superscript are considered statistically significant at $p < 0.05$. PW: Prostate weight

DISCUSSION:

Benign prostatic hyperplasia (BPH) is among the commonest urological abnormality affecting the aging male. The cause of the increase in prostatic volume is multifactorial, but current research has implicated hormonal aberrations. Clinical assessment of the patient is integral to determining the optimal treatment strategy. Therefore, in searching for alternative agent that will be cheaper and safer, attention has now been focused on the use of medicinal plants for the management of BPH. The result from the oral administration of the combination of *Imperata cylindrica* and *J. curcas*, roots extracts, clearly demonstrates their effects in the testosterone-induced BPH in rats.

The determination of phytochemical composition of any plant material is a major index of its medicinal potential. Flavonoids present in high concentration in *Jathropha curcas* root is a potent antioxidant and free radical scavenger and has been shown to protect cell membranes from damage [10]. *In vitro* studies have also shown that flavonoids have anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities [11, 12].

An increase was observed in ALP levels at the end of the 14 day in the group treated with the combined extract of *Imperata cylindrica* and *Jathropha curcas* roots 50 mg/kg b.w of the extract compared with the positive group, standard control and group treated with *Imperata cylindrica* roots extract 50 mg/kg b.w. The values of the liver function test depends on the specificity for damage as well as their sensitivity [11].

Although, serum levels of both AST and ALT become elevated when disease processes affect the liver integrity, ALT is the more liver specific enzyme and therefore generally more specific to changes in activity levels than AST [13]. ALP significantly ($p < 0.05$) decreased when all the treatment groups were compared with group 1. Even if there had been an elevation in ALP upon extract administration, it could still not have confirmed liver damage because according to Odutola [14], ALP and AST originate from different tissues such as the liver, bones, intestine and placenta.

Total bilirubin significantly ($p < 0.05$) decreased when groups 6,7 and 9 were compared with group 1 after 14 days of treatment. All these may show that the effect of the methanol extracts of *Imperata cylindrica* and *Jathropha curcas* roots extract in this study may not be toxic.

Table 5 showed no significant difference in urea and creatinine levels in the serum of rats with BPH treated for 14 days with *Imperata cylindrica* and *Jathropha curcas* roots extracts. Blood urea nitrogen (BUN) is the end product of protein metabolism. Urea concentration is elevated in kidney damage, excessive protein intake and low fluid intake. The normal creatinine level in the study suggest that these plant extract did not alter protein metabolism in the rats [15].

Malondealdehyde (MDA) is an end product derived from peroxidation of polyunsaturated fatty acids and related esters. In contrast to free radicals, aldehydes are relatively stable and there- fore able to diffuse within or out of the cell and to attack targets distant from the site of original free radical initiated events. Furthermore, MDA does not just reflect lipid peroxidation, but is also a by-product of cyclooxygenase activity in platelets, and persistent platelet activation is a common feature of many clinical syndromes associated with enhanced lipid peroxidation. Thus, measurement of MDA levels in plasma or serum provides a convenient *in vivo* index of lipid peroxidation and represents a noninvasive biomarker of oxidative stress often clinically employed to investigate radical-mediated physiological and pathological conditions [16]. Circulating MDA levels were found to be significantly higher in BPH patients than in healthy donors [17]. However, other works found circulating MDA levels in BPH patients similar to those in controls [18].

Malondialdehyde levels significantly decreased in the combined group in a dose dependent manner . Lipid peroxidation is a major mechanism of cell injury in tissues and organs subjected to oxidative stress that has been studied extensively [19] The control of lipid peroxidation is of special significance in biology because of its particular importance in relation to membrane damage [20]. Rodriguez *et al.* [21] identified the iron content of *Imperata cylindrica* as ferrihydrite and jarosite at a proportion of 50% respectively within roots, rhizomes and leaves of the plant. Iron is known to undergo fenton (reaction between ferrous iron and hydrogen peroxide) reaction by removal of one electron from molecular oxygen results in the formation of superoxide which often produces other ROS such as H_2O_2 and peroxynitrite $ONOO^-$ and hydroxyl radical [22]. Also increase in lipid peroxidation in group 4 and 5 could also be as a result of the low concentration of flavonoids, (2.53 ±

0.22). Group 6 and 7 significantly ($p < 0.05$) decreased in MDA levels when compared with group 5, this could be attributed to the high concentration of flavonoids, terpenoids and phenols present in *Jathropha curcas* roots extract. MDA levels was also significantly ($p < 0.05$) decreased in group 9 when compared with group 4 and 5, demonstrating a good combination effects of the both plant extract.

The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers [23]. *Jathropha curcas* roots extract with high flavanoids and phenols may act as a metal chelator in reducing the ferric state of iron in *Imperata cylindrica* to its ferrous state which is a more stable product and terminate lipid peroxidation. Superoxide dismutase activities (SOD) significantly ($p < 0.05$) increased in the combined group with 100 mg/kg b.w. Catalase activities also significantly ($p < 0.05$) increased in the combined group in a dose dependent manner.

The effects of administration of *Imperata cylindrica, Jathropha curcas* and combined extract on testosterone level showed a significant ($p < 0.05$) difference across the test groups. A decrease was observed in serum testosterone level when both extracts were combined, following the 14 days treatment. This decrease indicate the corresponding effects of the plant extract on total cholesterol lowering levels, since cholesterol is a possible substrate for testosterone synthesis and the presence of steroids in both plants prevented the absorption of cholesterol in the intestines. Zinc concentration in serum of BPH rats were observed in this study. The concentration of zinc significantly ($p < 0.05$) decreased when group 6 was compared with the other groups with exception of group 4. This indicates that, the roots extract of *Jathropha curcas* had a lower zinc level compared with *Imperata cylindrica* roots at the same dose of 50 mg/kg b.w tested. However, the combination of the two extracts significantly ($p < 0.05$) increased zinc concentration, indicating the potentials of both plants in management of BPH. This is in line with the work of Chyan *et al*. [24] who reported that at high tissue concentrations, this trace element inhibits the transformation of testosterone to dihydrotestosterone and plays an important role in maintaining the physiological function and normal tissue structure of the prostate.

Elevated levels of PSA are usually associated with prostate disorders such as BPH. A decrease in PSA is linked to a reduction in prostate hyperplasia due to inhibition of prostatic 5α-reductase. Several plants have been reported to have 5α-reductase inhibitory activity and hence prevent the development of BPH [25, 26, 27]. There is strong evidence that phytochemical agents are effective inhibitors of 5α-reductase that consequently leads to reduction in DHT concentrations and slows down BPH [28]. Hence treatment with *Imperata cylindrical* and *Jathropha curcas* roots extract showed a significant ($p < 0.05$) decrease.

Treatment with *Imperata cylindrical* and *Jathropha curcas* extracts significantly decreased the prostate weight in a dose dependent manner, indicating both plants potentials in management of BPH. Prostatic enlargement is used as an important marker for the disease. The prostatic index in this study significantly ($p < 0.05$) decreased in the combined groups. This confirmed the effects of the plants on prostate and body weight reduction observed in this study and also in line with the work of Bhavin *et al*. [29].

CONCLUSION:

The combined effects of methanol extracts of *Imperata cylindrica* (L) and *Jathropha curcas* (L) roots were found to be more effective in management of BPH, than the individual plants. This may be attributed to the phytochemicals of the plant as demonstrated in this study where combined extract was able to ameliorate the disease. This therefore suggests the potentials of the combined plant extracts for the management of BPH.

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