Nigerian Journal of Pharmaceutical and Applied Science Research, 5(2):34-40, August 2016

WEBSITE: www.nijophasr.com ***ISSN 1485-8059***

Cow Dung Extract Inhibited Testosterone-Induced Benign Prostatic Hyperplasia (BPH) In Wistar Rats

Olusegun S. Ajala*, Titilope A. Adelekan and Afolabi S. Lamina

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, CMUL Campus, PMB 12003, Surulere, Lagos, Nigeria.

ABSTRACT

Animal dungs could be a veritable source of steroidal and/or non-steroidal 5α-reductase inhibitory anti-Benign Prostatic Hyperplasia (BPH) drugs. A methanol cow dung extract was therefore evaluated for its possible anti-BPH activity *in vivo* in rats. Male Wistar albino rats (48) were randomly sorted into six groups of 8. Prostate hyperplasia was induced in five of the groups by a 30- day daily subcutaneous administration of 3mg/kg testosterone. One (the control) of these BPH model groups received a testosterone only regimen while each of the remaining four received orally the BPH-inducing testosterone concurrently with one of 100mg/kg, 200mg/kg, 400mg/kg cow dung extract or 10mg/kg finasteride (standard reference). The sixth group (bland) received olive oil and 2% tween 20, the respective vehicles for the hyperplasia-inducing subcutaneous testosterone and the remedial oral extract/finasteride. Each animal was sacrificed on the 31st day, its prostate removed, weighed and prostate weight/100g animal subsequently determined.Testosterone was found to produce a highly significant increase ($p < 0.0001$) in prostate weight after a 30-day daily administration. Likewise, cow dung extract was found to cause a highly significant inhibition ($p < 0.0001$) of the testosterone-induced prostate weight increase at 100mg/kg, 200mg/kg and 400mg/kg doses each. Cow dung could therefore be explored as a promising source of new 5α-reductase-inhibiting anti-BPH compounds.

Key words: Cow dung, Natural product drug discovery, Benign Prostatic Hyperplasia, 5α-reductase inhibitors.

INTRODUCTION

The Prostate is a walnut-sized gland lying below the bladder and surrounding the base of the male urethra. It produces the fluid in which spermatozoa are borne and nourished. It undergoes gradual enlargement, which may or may not be malignant, usually from the fourth decade of human life (Amis Jr., 1994). Benign Prostatic Hyperplasia (BPH) is the medical condition of the non-malignant enlargement of the prostate, causing painful urination or, in the worst of cases, outright cessation of urination (Bosch et. al., 1995). Autopsy studies have put the prevalence of histologic BPH at 50% in men between 50-60 years of age and 90% in men older than 80 years (Patel and Parson, 2014). Clinical prevalence, though a bit lower, is equally worrisome, as one-quarter of men in their 50s, one-third of men in their 60s and one-half of men in their 80s have been estimated to present with clinical symptoms (Kramer et. al., 2007; McVary et. al., 2011). In Nigeria, epidemiological data have shown that one-in-four men older than 40 years have symptoms suggestive of BPH (Ezeanyika et. al., 2006). If untreated or improperly managed, BPH could progressively result in bladder dysfunction, urinary retention, sepsis, toxaemia and eventual death (Chapple et. al., 2008; Roehrborn et. al., 2008). Macroscopic enlargement of the prostate, upon which clinical BPH is consequent, has been attributed to many aetiological factors, the leading amongst which is shift in prostatic androgen metabolism causing intraprostatic accumulation of dihydrotestosterone (DHT) (Isaacs, 1993). Therefore, producing animal models of the disease has largely been by an indirect simulation of prostatic accumulation of DHT by the elevation of circulating testosterone.

* Corresponding author: Email- olusegunajala@yahoo.co.uk Phone+234 (0) 803 562 2151

Moreover, the central role that prostatic testosterone metabolism plays in BPH development is evident in the fact that the only pharmacotherapeutic strategy that does not only relieve BPH's associated Lower Urinary Tracts Symptoms (LUTS), but also reverses or halts the progression of the disease, is the inhibition of the enzyme 5α-reductase, which converts testosterone to DHT in the prostate gland (Hiebble, 2004; Fitzpatrick and Artibani, 2006).

There is however a paucity of 5α-Reductase inhibitors in clinical medicine; the available two (finasteride and dutasteride) are also associated with severe depressive and sexual side effects (Irwig and Kolukula, 2011; Irwig, 2012a, Irwig, 2012b), indicating inhibition of testosterone at macromolecular binding sites other than the intraprostatic 5α-Reductase. There is therefore an urgent need for the discovery of new 5α-Reductase Inhibitors (5ARIs) with better 5α-Reductase inhibition selectivity profile, a biochemical attribute that would require intricate structural novelties mostly, if not exclusively, found in nature-derived compounds (Koehn and Carter, 2005; Keller et. al., 2006; Newman and Cragg, 2012).

Animal dungs represent one rather unexplored natural resource of bioactive compounds with high propensity of structural and functional novelties. This probably accounts for their historical ethnomedicinal uses, including the diverse uses of the excreta of white pigeon and Trogopterus in Traditional Chinese Medicine, use of crocodile excreta as spermicides in ancient Egyptian Traditional Medicine and use of cow dung for various purposes in Ayurvedic medicine (Yang et. al., 1987; Mull et. al., 1990; Basak et. al., 2002; Mandavgane et. al., 2005; Aboesoud, 2010). Animal faeces or dungs are the ultimate destination of the steroidal and non-steroidal products of secondary metabolism of animal gut microbial commensals. More structurally diverse bioactive molecules could be further speculated to be present in animal dungs courtesy of the biotransformation activities of the afore-mentioned gut microbes on the carbocyclic steroidal skeleton of bile acids that escape enterohepatic reabsorption (Rosenthal and Glew, 2009), and bioactive molecules from dietary sources (Nicholson et.al., 2012; Sayin et. al., 2013).

The steroidal components of animal dungs could competitively inhibit the steroidal natural substrate of 5α-Reductase, i.e., testosterone, at the enzyme's active site. The intrinsic DNA interactive nature of steroid activity (Fullerton, 1998) could also portend inhibitory influence of the steroidal components on 5α-Reductase via interference with the enzyme's expression. On another hand, allosteric interaction of the non-steroidal components of animal dungs could portend non-competitive inhibition of the enzyme. By and large, therefore, animal dungs could be conjectured as a repository of diverse steroidal and non-steroidal molecules with high tendencies of 5α-Reductase inhibition.

In the light of the above, we chemically profiled the methanol extract of a sample of cow (*Bos taurus*) dung collected from a public abattoir in South West Nigeria and subjected it to anti-BPH evaluation by monitoring its inhibitory effects on testosterone-induced prostate weight increase in male albino rats.

MATERIALS AND METHODS

Materials

Fresh cow dung was obtained from Ilaje abattoir in the Bariga Local Government area of Lagos State, Nigeria, and sun-dried. Animals for the animal experiments were obtained from the University of Lagos College of medicine animal house. All animal procedures conformed to the guidelines of the Experimental Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Nigeria, and were in accordance with the UK animal (scientific procedure) act, 1986. Testosterone Enanthate 250mg/ml (Rotemedica, Germany) and finasteride 5mg (Teva, UK) were obtained from a local pharmacy in the neighbourhood of the College of Medicine of the University of Lagos. All solvents were obtained from Aldrich and were of, at least, analytical grade.

Methods

***Extraction*:** Cow dung (3kg) was extracted under reflux conditions with methanol (2L) for 3hours and 1.5hours successively. The extracts were combined and evaporated to dryness *in vacuo* at 40°c.

***Chemical profiling*:** Standard phytochemical screening methods as described by Trease and Evans (2002) were employed in chemically evaluating the cow dung extract for steroidal and other pharmacologically important bioactive chemical contents such as alkaloids, flavonoids, cardiac glycosides, terpenes, etc.

Evaluation of cow dung extract effect on testosterone-induced BPH

Forty-eight male Wistar albino rats, weighing between 80 - 150g, were obtained and housed in the animal care centre, College of Medicine University of Lagos, Nigeria. The rats, maintained under standard conditions, had unrestricted access to feed (Pfizer mouse cubes) and clean water *ad libitum*. The animal room was well ventilated and maintained at a temperature range of 25°C-27°C.

The rats were randomly divided into 6 groups of 8. Group 1(the control) was treated with subcutaneous testosterone (3mg/kg) dissolved in olive oil concurrently with 10mg/kg 2% tween 20 administered orally. Groups 2, 3, 4 and 5 were treated with testosterone (3mg/kg) dissolved in olive oil subcutaneously and one of 100mg/Kg, 200mg/Kg, 400mg/Kg cow dung extract or 10mg/kg finasteride, each suspended in 2% tween 20 and administered orally. Group 6 (the bland group) was treated with olive oil subcutaneously and 2% tween 20 orally and set as control for the assessment of testosterone-induced BPH. All groups received their respective treatments simultaneously once daily for 30 days. The animals were fasted overnight prior to the 31st day, on which they were sacrificed using diethyl ether anaesthesia. The prostate of each animal was removed, weighed and mean weight per 100g animal for each group calculated.

Statistical analysis of data

All biological data were expressed as Mean ± SEM. Evaluation of the hyperplasia inducing effect of testosterone on the prostate was done by t-test. One way Analysis of Variance (ANOVA) followed by Tukeys' multiple comparison test (TMCT) was used to evaluate the prostatic hyperplasia inhibitory effects or otherwise of the various concentrations of the cow dung extract and the standard drug finasteride. Confidence interval was placed at 95%, so that in all cases, a value of $p < 0.05$ was considered significant. These analyses were performed using Graphpad prism software, version 6 (Graphpad software, Inc., La Jolla, CA, USA).

RESULTS

Chemical evaluation using standard phytochemical screening methods showed the cow dung extract as containing steroids, saponins, flavonoids and terpenoids. Table 1 shows a summary of the results of this chemical evaluation. Mean prostate weight/100g animal for the six experimental groups were obtained and are as displayed in Table 2.

Table 1: Phytochemicals found in cow dung extract.

Phytochemicals	Availability in cow dung extract
Flavonoids	+
Tannins	-
Saponins	+
Cardiac glycosides	-
Volatile oils	+
Alkaloids	-
Steroids	+
Terpenes	+
Anthraquinones	-

Key: + = Present, - = Absent

Table 2: Effect of cow dung extract on the Mean prostate weight/100g animal.

Experimental group	Mean prostate weight/100g animal±SEM
Bland	0.09±0.007*
Testosterone only	0.25±0.016
100mg/Kg extract	0.09±0.007*
200mg/Kg extract	0.09±0.004*
400mg/Kg extract	0.08±0.010*
Finasteride	0.06±0.009*

*highly significantly different from the control ($p < 0.0001$)

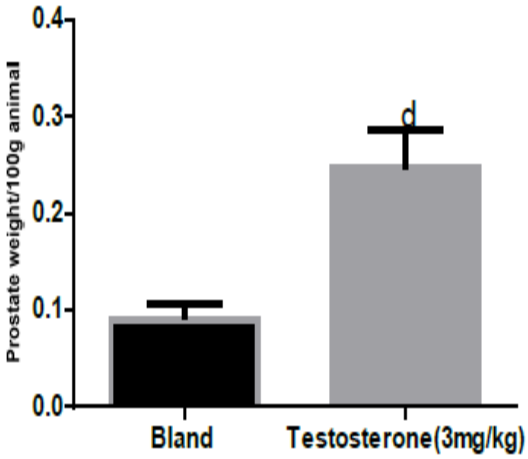
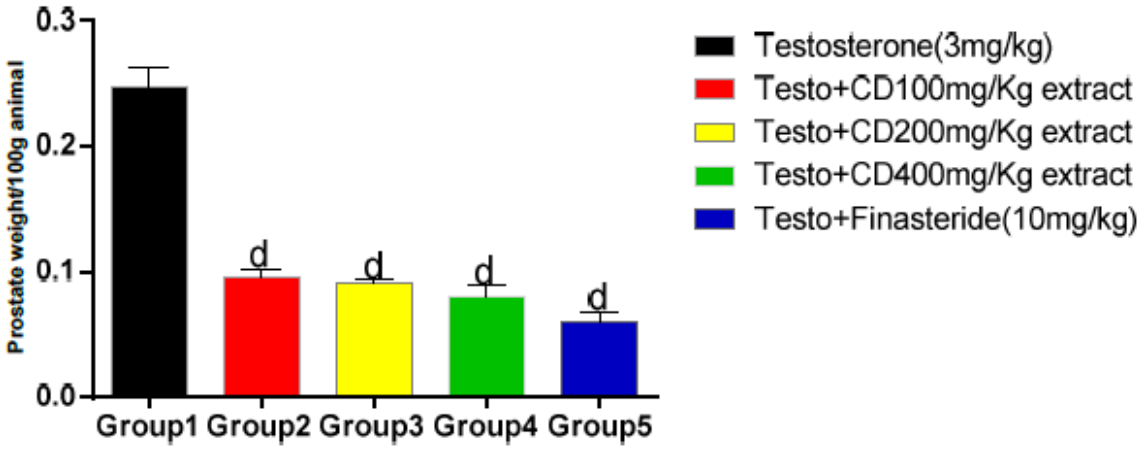


Figure 1: A t-test comparison of the mean prostate weight/100g animal of the bland and testosterone only-treated groups.

d= highly significant increase ($p < 0.0001$) in mean prostate weight/100g animal compared to the bland (2% tween 20 and olive oil) group.



Key: Testo = Testosterone; CD = Cow dung

Figure 2: One way ANOVA comparison of the mean prostate weights/100g animal of the testosterone only-treated group (group 1) and the 100mg/kg extract-, 200mg/kg extract-, 400mg/kg extract- and fiasteride-treated groups (groups 2, 3,4 and 5 respectively).

d = highly significantly reduced ($p < 0.0001$) mean prostate weight/100g animal compared to the testosterone only group.

Hyperplasia-inducing effects of testosterone on the prostate was evaluated by a t-test comparison of the mean prostate weight/100g animal of the testosterone only group with that of the bland as depicted in Figure 1, which shows a highly significant higher prostate weight ($p < 0.0001$) for the testosterone only group than the bland after the 30-day treatment. In the same vein, testosterone-induced hyperplasia inhibitory effects or otherwise of three concentrations of the cow dung extract and the standard drug, finasteride, were evaluated by a one way ANOVA comparison of the mean prostate weight of the testosterone only group with those of the rest as depicted in figure 2, which shows that there was a highly significant decrease ($p < 0.0001$) in the mean Prostate weight /100g animal of each of the 100mg/kg-, 200mg/kg-, 400mg/kg- and finasteride-treated groups compared to the (untreated) testosterone only group.

DISCUSSION

The choice of cow dung as animal dung for this study was more for quantitative than qualitative reasons: In addition to the ready availability of cow dung in large quantities, the cow's copious production of bile, evident in the dark-green colour of cow dung, is indicative of a large amount of bile and, hence, of bile salts and their presumed steroidal metabolites in the cow dung.

Chemical profiling of cow dung for possible presence of bioactive compounds is an indirect approach to the exploration of the diversity of the products of metabolic activities of cow gut's microbial commensal community. This molecular diversity, as earlier speculated, is a composite of the secondary metabolites of the intestinal microbes and the products of their unique biotransformation activities on the cow's steroidal bile components (Mahato and Garai, 1997) and diet-derived xenobiotics. Chemical profiling results revealed the presence of steroids, saponins, terpenoids and flavonoids (Table 1) in the cow dung extract. While the steroidal components could be presumed to be bile acids/salts , their microbial metabolic products and the steroidal components of the products of the microbes' secondary metabolism, the saponin contents might have come from enzymatic conjugation of some of these steroids with oligosaccharides and unabsorbed monosacharides in the intestinal lumen. The flavonoid and terpenoid contents could be largely ascribed to dietary sources and the secondary metabolism of the intestinal microbes. Nevertheless, diet-derived components, by virtue of having come under microbial metabolic transformation, would most likely possess unique structural features that would make them different from those present in the ingested plant diet (Ilett et. al., 1990). Though it is possible that the extract's chemical diversity is actually more than the adopted phytochemical methods could detect, the identification of four bioactive chemical groups (steroids, steroidal analogue saponins, flavonoids and terpenoids), with established diverse bioactivity pedigrees (Bhat et. al., 2005), is enough scientific platform to speculate that cow dung extract contains 5α-reductase inhibitors with possible active and/or allosteric site-binding mechanisms (Silverman, 2004), and thereby rationalizing its observed anti-BPH activity.

The mean prostate weight of the testosterone only-treated group at the end of the 30-day treatment was significantly higher ($p < 0.5$) than that of the bland which received the vehicles of the tumour-inducing testosterone and the test/standard remedies. This shows that testosterone is capable of inducing BPH as also have been reported by a number of earlier investigators, though with varying doses and duration (Gossel-Williams et. al., 2006).

The mean prostate weight/100g animal of each of the 100mg/kg, 200mg/kg and 400mg/kg extract-treated groups was highly significantly lower ($p < 0.0001$) than that of the testosterone only-treated group and comparable with that of that of the standard reference (finasteride). This indicates a significant inhibition of the testosterone-induced BPH by the three doses of the cow dung extract used. The dose dependency of this inhibition is not apparent, suggesting that the inhibition was either non-competitive or saturated, if competitive. Further studies would thus be needed to unravel the competitive nature or otherwise of 5α-Reductase inhibition by the extract.

Because the inhibited BPH was testosterone-induced, the anti-BPH mechanism of the extract as 5α-Reductase inhibition is unequivocal (Pelletier et. al., 1998). The yet-to-be-unravelled competitive nature of the inhibition, however, makes competitive and allosteric inhibition as well as inhibition of enzyme expression possible options, thereby making speculations on the chemical structure of the anti-BPH components of the extract, at this stage, difficult.

In conclusion, a testosterone-induced Benign Prostatic tumour was significantly inhibited by oral cow dung extract at 100mg/kg, 200mg/kg and 400mg/kg doses, inferring 5α-reductase inhibition. Cow dung is hereby recommended as a promising

source of new 5α-reductase inhibitory anti-BPH agents.

REFERENCES

Aboelsoud NH (2010). Herbal medicine in ancient Egypt. *Journal of Medicinal Plants Research*. 4(2):082-086

Amis Jr ES (1994). Anatomy and Physiology of the Prostate In: Radiology of the Lower Urinary Tract Springer Berlin Heidelberg pp. 167-169

Basak AB, Lee MW and Lee TS (2002). In vitro inhibitory activity of cow urine and dung to *Fusarium solani* f. sp. cucurbitae. *Mycobiology* 30(1):51-54

Bhat SV, Nagasampagi BA and Sivakumar M (2005). Chemistry of natural products. Narosa Publishing House, New Delhi pp. 15-235, 585-660

Bosch JR, Hop WC, Kirkels WJ and Schröder FH (1995). Natural history of benign prostatic hyperplasia: appropriate case definition and estimation of its prevalence in the community. *Urology* 46(3):34-40

Chapple CR, Wein AJ, Abrams P, Dmochowski RR, Giuliano F, Kaplan SA, McVary KT and Roehrborn CG (2008). Lower urinary tract symptoms revisited: a broader clinical perspective. *European urology* 54(3):563-569

Ezeanyika LU, Ejike CE, Obidoa O and Elom SO (2006). Prostate disorders in an apparently normal Nigerian population 1: Prevalence. *Biokemistri*18(2):127-132

Fitzpatrick JM and Artibani W (2006). Therapeutic strategies for managing BPH progression. *European urology supplements* 5(20):997-1003

Fullerton DS (1998). Steroids and Therapeutically related compounds. In: Delgado JN and Remers WA (eds). Textbook of organic medicinal and pharmaceutical chemistry. 10th edition, Lippincott-Raven Publishers, Philadelphia, USA, pp. 727-731

Gossel-Williams M, Davis A and O'Connor N (2006). Inhibition of testosterone- induced hyperplasia of the prostate of Sprague-Dawley rats by pumpkin seed oil. *Journal of medicinal Foods* 9(2):284-286

Hieble JP (2004). Therapeutic strategies for benign prostatic hypertrophy. Drug *Discovery Today: Therapeutic Strategies* 1(2):243-248.

Ilett KF, Tee LB, Reeves PT, Minchin RF (1990). Mebolism of drugs and other xenobiotics in the gut lumen and wall. *Pharmacology & therapeutics* 46(1):67-93

Isaacs JT(1993). Etiology of benign prostatic hyperplasia. *European urology* 25:6-9

Irwig MS(2012a). Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects. *The Journal of clinical psychiatry* 73(9):1220-1223

Irwig MS (2012b). Persistent sexual side effects of finasteride: could they be permanent? *The journal of sexual medicine* 9(11):2927-2932

Irwig MS and Kolukula S (2011). Persistent sexual side effects of finasteride for male pattern hair loss. *The journal of sexual medicine* 8(6):1747-1753

Keller TH, Pichota A and Yin ZA (2006). practical view of 'druggability'. *Current opinion in chemical biology* 10 (4):357-61

Koehn FE and Carter GT (2005). The evolving role of natural products in drug discovery. *Nature reviews Drug discovery* 4(3):206-20

Kramer G, Mitteregger D and Marberger M (2007). Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *European urology* 51(5):1202-1216

Mahato SB, Garai S (1997). Advances in microbial steroid biotransformation. *Steroids* 62(4):332-45

Mandavgane SA, Pattalwar VV and Kalambe AR (2005). Development of cow dung based herbal mosquito repellent. *Natural product radiance* 4(4):270-272

McVary KT, Roehrborn CG, Avins AL, Barry MJ, Bruskewitz RC, Donnell RF, Foster HE, Gonzalez CM, Kaplan SA, Penson DF and Ulchaker JC (2011). Update on AUA guideline on the management of benign prostatic hyperplasia. *The Journal of urology* 185(5):1793-803

Mull DS, Anderson JW and Mull JD (1990). Cow dung, rock salt, and medical innovation in the Hindu Kush of Pakistan: the cultural transformation of neonatal tetanus and iodine deficiency. *Social Science & Medicine* 30(6):675-91

Newman DJ and Cragg GM (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of natural products* 75(3):311-35

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W and Pettersson S (2012). Host-gut microbiota metabolic interactions. *Science* 336(6086):1262-1267

Patel ND and Parsons JK (2014). Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. *Indian journal of urology* 30(2):170-176

Pelletier G, Luu-the VA, Huang XF, Lapointe H and Labrie F (1998). Localization by in situ hybridization of steroid 5 alpha-reductase isozyme gene expression in the human prostate and preputial skin. *The Journal of urology* 160(2):577-82.

Roehrborn CG, Siami P, Barkin J, Damião R, Major-Walker K, Morrill B, Montorsi F and Comb AT (2008). The effects of dutasteride, tamsulosin and combination therapy on lower urinary tract symptoms in men with benign prostatic hyperplasia and prostatic enlargement: 2-year results from the CombAT study. *The Journal of urology* 179(2):616-21

Rosenthal MD and Glew RH (2009). Medical Biochemistry, Human metabolism in health and disease. John Wiley, Hoboken, NJ. USA, pp.48-49

Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell metabolism* 17(2):225-35

Silverman RB (2004). The organic chemistry of drug design and drug action. 2nd edition. Elsevier Academic Press. London, UK, p. 242

Trease GE and Evans WC (2002). Pharmacognosy. 15th Ed. London, Saunders Publishers, pp. 42–44, 221-229

Yang DM, Su SW, Li x, Zhu TR (1987). Studies on bioactive constituents from the excreta of *Trogopterus xanthipes* Milne . *Acta Pharmaceutica Sinica* 22(10):756-60