

Inhibitory effect of *Coffea arabica* bean in testosterone induced prostatic hyperplasia in Sprague-Dawley rats

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**Kristian Alfonso G. Cueto, Jenn Andrea C. Macalincag,
Rhoneelet B. Aseremo, Joanne Florence B. Gutierrez,
Sarah Jane R. Mabiog, Carina R. Magbojos and
Oliver Shane R. Dumaoal**

College of Allied Medical Professions, Lyceum of the Philippines
University, Capitol Site, Batangas City, Philippines

¹Corresponding Author: kristianalfonsocueto@yahoo.com

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Abstract: Benign prostatic hyperplasia (BPH) has been described as the uncontrolled prostate gland growth which leads to difficulty in urination. One of the treatment of BPH is saw palmetto lipid extracts which has been shown to inhibit prostate 5 α -reductase and some of its components (lauric acid, myristic acid and oleic acid) also inhibit the enzyme. Coffee was also rich in fatty acids namely linoleic acid, oleic acid and palmitic acid. The aim of this research is to investigate whether coffee is effective in preventing testosterone-induced prostatic hyperplasia in rats using testosterone propionate and estradiol valerate. After and before the induction, the rats were tested for prostate specific antigen (PSA). The condition of the prostate gland of the test animals were correlated with the results of the said test and in the histopathologic results. After 14 days of experimentation, animals in the test group significantly decreased their PSA levels as compared to the BPH group. The histomorphology showed that *Coffea arabica* bean oil inhibited testosterone propionate while estradiol valerate induced prostatic hyperplasia. These findings indicate that *Coffea arabica* bean oil effectively inhibited the development of BPH. With the proven safety of coffee oil, these findings strongly support the feasibility of using *Coffea arabica* bean oil therapeutically in treating BPH.

Keywords: coffee bean, fatty acids, benign prostatic hyperplasia

INTRODUCTION

BPH is the fourth most common disorder associated in elderly men and is also the most common cause of lower urinary tract symptoms [1]. LUTS is associated with incomplete bladder emptying, weak urinary stream, dysuria, nocturia and bladder outlet obstruction. BPH is the increase in the size of the gland due to the proliferation in total number of stromal cells and epithelial cells which leads to a significant impairment of day to day functioning and quality of life. It is associated with distinct alteration in tissue histomorphology [2]. Although it is benign disease, it can cause problems on the quality of life of elderly men [3]. The enlarged prostate impinges on the urethra that leads to the impairment in urinary function [4]. Its etiology is still largely unresolved. However, it seems that the pathologic mechanism is endocrine controlled and

involves alterations in the metabolism of androgens and estrogens [5]. Inflammatory features including infiltration of activated T cells, macrophages, and mast cells capable of secreting growth factors and proinflammatory cytokines are exhibited by about 40% of BPH patients [6].

BPH is inhibited through different plant oils containing fatty acids. Fatty acids like myristic acid, linoleic acid, linolenic acid, oleic acid, lauric acid, stearic acid and others are some of those with clinical significance.

Coffee bean is the seed of the coffee plant and one of the most economically important variety of this is the Arabica which represents 75-80% of the coffee produce worldwide. There are 6% w/w total fat in coffee. It is composed of fatty acids that can be a candidate for the inhibition of BPH. D-004 which contains fatty acid like linoleic acid is said to inhibit

5- α reductase in vitro and decreases testosterone induced prostatic hyperplasia in rodents (Fernandez, Fernandez, Gamez, Hollands, Illnait, Jimenez, 2009). Fatty acids together with vitamin E and tocotrienols are included with the most prominent anticarcinogens (Cosgrove, Kaina, Mosby, Plati and Sarkadel, 2012). Linoleic acid is one of the major constituents of saw palmetto extract which is widely used for the treatment of benign prostatic hyperplasia [1].

In animals, BPH can be induced through the use of different hormones. One of these is dihydrotestosterone (DHT) is a biologically active metabolite of the hormone testosterone. It belongs to the class of compounds called androgens. It is purely androgenic and cannot be transformed into estrogen [4]. DHT is thought to be approximately 30 times more effective than testosterone because of increased affinity for the androgen receptor. Although, testosterone could also be a factor in inducing BPH [7]. DHT also play a role in the worsening of BPH and prostate cancer. DHT is important in the development of secondary sex characteristic and sex differentiation [8]. Estradiolvalerate is absorbed more slowly and possesses a longer duration, especially when given in an oil solution by intramuscular injection and can be administered less frequently.

Borst, Carter, Conover, Gregory, Leeuwenburgh, Marzetti [9] revealed that 5 α -reductase is responsible for the conversion of testosterone to dihydrotestosterone. Wherein, 5 α -reductase inhibitor will inhibit the development of BPH by reducing DHT [10].

As of now there is no completely effective treatment for BPH [11]. The treatment of BPH is a significant public health issue, as a large number of men will be exposed to the morbidity associated with BPH and its various modalities. According to recent studies, it is said that fatty acids are responsible for the inhibition of BPH. Our study wants to prove that fatty acids of coffee can inhibit BPH. It aims to lessen the number of men having the said illness.

MATERIALS AND METHODS

Preparation of Coffee bean

Dried coffee bean was grind until it was almost pulverized. The moisture content of the ground coffee bean was computed, it was computed by subtracting the initial weight and its weight after putting it in the oven for overnight.

Extraction of oil from Coffee

Fifty grams of ground coffee bean is dissolved in 250 ml of petroleum ether in an Erlenmeyer flask, agitated in a mechanical rotator for 8 hours. Aspirate the supernate and repeat the extraction twice. Collect all the supernate and place it in a vial in equal amount. Then place all the vials in a fume hood for the evaporation of the petroleum ether and obtaining the coffee oil.

Preparation of laboratory animal

Sixteen 4 weeks old Sprague Dawley rats weighing 200-250g housed in a cage at 25C with 12 hours light/dark cycles. They were divided into four equal group - one test group and three control groups. The test group will receive 200mg/kg of coffee oil once a day through oral gavage and subcutaneous injections of 0.2ml of testosterone propionate and estradiol valerate (ratio 10:1) in the leg part every day for 14 days. The first control group received the hormones, but without coffee oil. The second control group, received subcutaneous injection of 0.2 ml of testosterone propionate and estrogen valerate and oral gavage of coffee oil. The third control group was injected subcutaneously with 0.2 ml of olive oil but without coffee oil [12].

Biochemical tests

Blood samples obtained from rats of the same species, same age and same body weight will be assayed for prostate specific antigen (PSA). It will be measured in the serum of the test animal of various groups using the PSA ELISA kit following the procedure that will be supplied with the kit. It will be tested before and after the experimentation which will be then compared with the result of the pre-test and will be evaluated.

Histopathology

After 14 days, the rats were subjected for tissue biopsy. Rat's prostate gland will be removed and fixed overnight and processed into paraffin and 5 μ m sections will be stained with hematoxylin and eosin (H and E) or Gomori's trichrome to evaluate collagen. Histopathological evaluation will be blinded and performed by a trained veterinary pathologist [7].

Statistical Tool

All results were expressed as the mean \pm SEM. Statistical analyses will be performed using analysis of variance, comparison between negative and

positive groups for the data is to be made by using one way analysis of variance. The difference between the pretest and posttest were compared with t-test.

RESULTS AND DISCUSSION

The coffee oil was subjected to Gas Chromatography. It was done by the Department of Science and Technology, Industrial Technology Development Institute Standards and Testing Division (DOST-ITDI). Fatty acids of some extracts was embedded in the oils of some plants. The coffee oil composition, specifically the fatty acids content can be considered as chemical descriptor to differentiate between coffee varieties (Hurtado and Durado, 2013). The extraction of oil was done with the use of highly volatile solvent.

Table 1 shows the fatty acid profile done by DOST-ITDI. The determination showed that the predominant fatty acid in the coffee oil was linoleic acid with 74.5%.

This implies that the coffee oil contains fatty acids which are the component of choice for the study. The four dominant fatty acids are linoleic, palmitic, stearic and oleic acid.

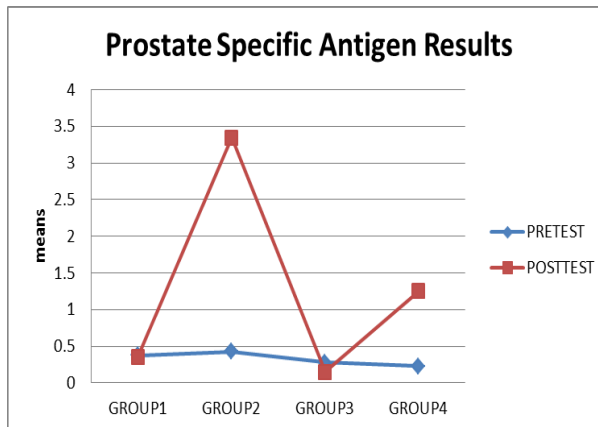


Figure 1. Difference Between PSA posttest and pretest.

PSA serum levels are said to be abnormally elevated in patients with prostate cancer, BPH and patients with inflammation of prostate [13]. Figure 1 presents the difference between the PSA pretest and posttest. The Group 1 showed no significant difference between the two tests. It is brought by the treatment (coffee oil) given to the test group that inhibits the conversion of testosterone to dihydrotestosterone.

Table 1. Fatty Acid Profile of Coffee Oil

Fatty acid	Result	Fatty acid	Result
a. Total Fat, % w/w	6	Heptadecanoic (C17)	ND
b. Fatty Acid Profile, % w/w		Stearic (C18)	4.86
Caproic (C6)	ND	Oleic (C18:1)	3.48
Heptanoic (C7)	ND	Linoleic (C18:2)	74.5
Caprylic (C8)	ND	Linolenic (C18:3)	0.152
Nonanoic (C9)	ND	Eicosanoic Acid (C20:1)	ND
Capric (C10)	ND	Nonadecanoic (C19)	ND
Lauric(C12)	ND	Archidic (C20)	0.926
Tridecanoic (C13)	ND	Eicosapentanoic (C20:5)	ND
Myristic (C14)	ND	eneicosanoic (C21)	ND
Pentadecanoic (C15)	ND	Behenic (C22)	0.297
Palmitic (C16)	15.8	Docosahexaenoic (C22:6)	ND
Palmitoleic Acid (16:1)	ND	Tricosanoic (C23)	ND
		Nervonic (C24:1)	ND

In contrast to the Group 1, Group 2 there is significant difference between the pretest and posttest done. This result implies that the induction of testosterone without treatment produces the prostate cells to proliferate that made the PSA posttest level be elevated. The Group 3 exhibited a decrease in PSA level in the posttest. It is associated to the response of the rat's body to the testosterone. It is connected to the study of Dixit and Nahata [13], which tells that if there is a decrease in PSA levels is observed, it can be assessed that the test group in question is having protective effects on inflammatory condition and hypertrophy of the prostate induced by testosterone. There is also an increase with the result of the pretest and posttest of the vehicle group, given only by subcutaneous injection of olive oil.

Figure 2A illustrates the shrinkage of prostate glands devoid of cells but with gel-like content. This indicates that coffee oil was able to inhibit the testosterone activity in the prostate of the rats. The fatty acids present in the coffee oil are 5- α reductase inhibitors such as palmitic, oleic, linoleic and stearic acid. Those fatty acids are targeted against the enzyme 5- α reductase which is responsible for the conversion of testosterone to dihydrotestosterone [7] Based on these results, coffee oil treatment effectively

obstructed the prostatic hyperplasia in rats induced by testosterone.

It is demonstrated in Figure 2B that there is classical prostate hyperplasia with plenty of proliferating glands filled up with cells and gel-like content. This implies that testosterone efficiently induced prostatic hyperplasia in rats. The tubules became wider compared with the control. The walls of the tubules were thickened and every tubule almost had developed large involutions projecting into the lumen, reducing the volume of the lumen compared with the control [13].

In Figure 2C, enlarged hypertrophic glands without cells revealed but filled up with gel-like content. There were some mechanisms of testosterone that might have caused hyperplasia and hypertrophy. According to Nahata and Dixit [13], hypertrophy of the prostate can be induced by testosterone as one of the mechanisms of the rats' bodies is it.

Figure 2D presents shrinkage of glands without cells and scanty amount of gel-like content. This indicates that there were no changes in the vehicle group. These results agrees with Arruzazabala, Perez, Ravelo, Molina, Carbajal, Mas [14] upon the addition of the vehicle there was no change in the basal tone of the isolated prostate gland.

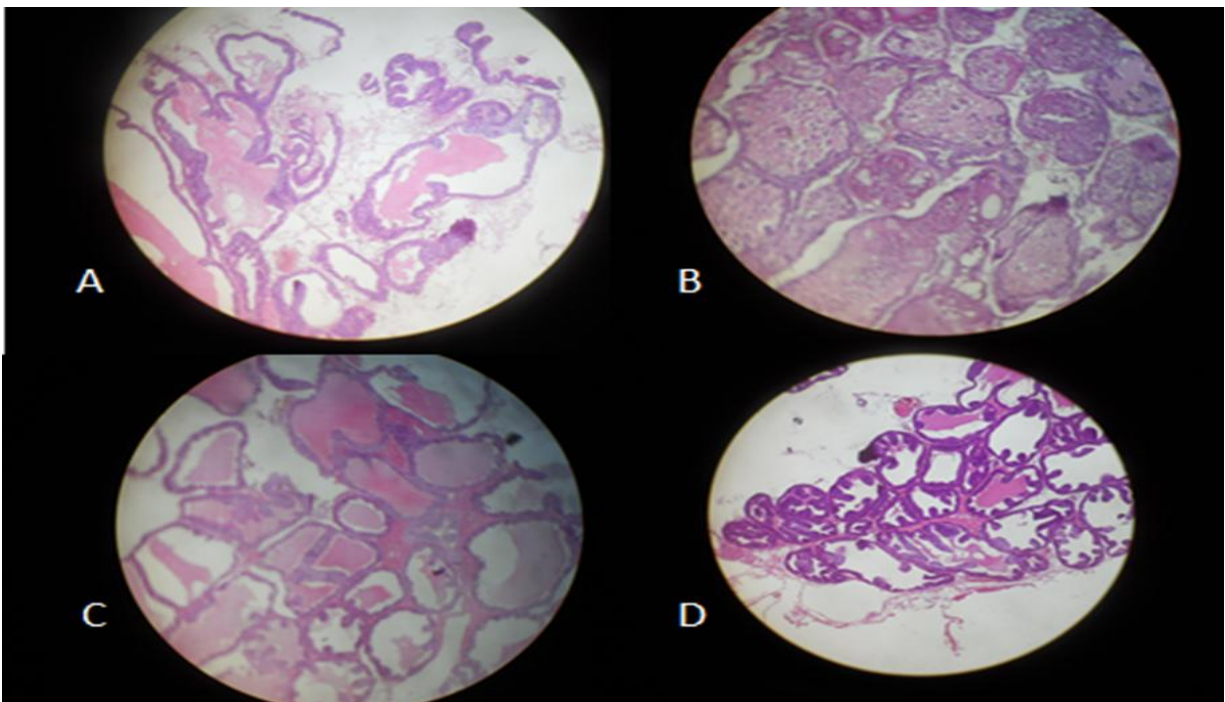


Figure 2 Histopathologic sections of rat prostate tissue from Group A (coffee oil and testosterone), Group B (testosterone only), Group C (DMSO and testosterone) and Group D (olive oil only).

Both the results of the PSA and histopathological examination showed the significant difference between the test group and the group 2 which only received subcutaneous injection of testosterone. Dihydrotestosterone (DHT), a steroid hormone from testosterone by the enzyme 5 alpha reductase, is the primary active metabolite of testosterone. The role of DHT in BPH is well known, as it is the androgen responsible for prostate growth. Therefore, DHT is ultimately responsible for prostatic epithelial and stromal cell hyperplasia or even hypertrophy in some rats. As mentioned above, because DHT is formed from testosterone by 5 alpha reductase, many studies have focused on reducing DHT level by inhibiting this enzyme. In the present study, coffee oil treated animals showed significant difference between the PSA results of the test group and BPH group. These results were consistent with the changes in prostate histopathological morphology. These findings indicate that *Coffea arabica* bean oil suppressed the development of BPH in the animal model by inhibiting the 5 alpha reductase enzyme.

CONCLUSION

In conclusion, fatty acids from coffee oil significantly lower the PSA posttest result of the test group as compared to the group with testosterone only. It was supported by the results of the histopathological examination that reveals that in the test group there was no proliferation of cells unlike in the group 2. These results indicate that *Coffea arabica* bean oil inhibits the development of BPH. Combined with proven safety of coffee oil, these findings strongly support the feasibility of using *Coffea arabica* bean oil therapeutically in treating BPH.

RECOMMENDATION

Further analysis is recommended to confirm the effect of the drug on BPH model. The use of roasted coffee bean was recommended for future researchers to assess the potency of coffee oil coming from roasted bean and also to evaluate the effects of simply drinking coffee in BPH.

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