Full Length Research Paper

Combination of lauric acid and myristic acid prevents benign prostatic hyperplasia (BPH) symptoms in animal model

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Benign and uncontrolled growth of prostate gland is known as benign prostatic hyperplasia (BPH). It is a common health issue that affects 8% of all men at the age of 40, 60% of men in their 70s, and 90% of those greater than 80 years of age. In this study, we investigated whether a combination of lauric and myristic acid improved BPH in a testosterone propionate (TP)-induced model of BPH in rats. BPH was induced in the rats with a subcutaneous injection of TP (3 mg/kg) and combination of different doses of lauric acid and myristic acids given every consecutive day for 4 weeks. Combination of lauric and myristic acid led to significant reductions in prostate weight and dihydrotestosterone levels in the serum and prostate. Therefore, combination of lauric acid and myristic acid was effective in reducing TP-induced BPH in a rat model, and may be useful for the clinical treatment of patients with BPH.

Key words: Testosterone propionate, benign prostatic hyperplasia, testosterone, dihydrotestosteone.

INTRODUCTION

Benign prostate hyperplasia (BPH) is a urological disorder caused by the noncancerous enlargement of the prostate as men age. As the prostate enlarges, it can constrict the urethra, inducing various symptoms including a weak urinary stream, incomplete bladder emptying, nocturia, dysuria and bladder outlet obstruction (Pais, 2010; Roehrborn, 2011). It is proliferative process of both stromal and epithelial elements of the prostate (McNeal, 1983). It is unclear what specific factors

regulate the degree of hyperplasia, which ultimately dictates the size of the prostate gland, there are many consensus regarding the prostate size that qualifies for the diagnosis of benign prostatic enlargement (BPE). As men age, the caliber of the urinary stream diminishes (Girman et al., 1995). The diminution of the urinary stream was assumed to be attributable to bladder outlet obstruction (BOO) arising directly from the BPE (Lepor, 2000). Androgens may be involved in the epithelial

stroma interaction. In mature prostate, androgens are known to cause several changes in prostatic epithelium through androgen receptors located in the stroma. Immunocytochemical studies have shown that prostatic smooth muscle cells are uniformly androgen receptor positive. This fact indicates that smooth muscle located in prostatic stroma may be an important target for androgen action and able to regulate the expression of prostate growth factors (Niu et al., 2003).

Although the etiology of benign prostatic hyperplasia is not completely elucidated, it involves hormonal changes in the aging man. The development and growth of prostate gland depends on androgen stimulation, mainly by dihydrotestosterone (DHT), an active metabolite formed due to enzymatic conversion of testosterone by steriod 5 α-reductase.

Production and accumulation of DHT in the prostate increases with ageing which results in encouraging cell growth and induction of hyperplasia (Carson and Ritterman, 2003; Bartsch et al., 2000). Benign prostatic hyperplasia also involves augmented adrenergic tone in prostate smooth muscles, regulated through α_1-adernoceptors (Michel et al., 1998).

Conventionally used drugs like 5 α-reductase inhibitors (finasteride and dutasteride), α-adrenoceptors antagonists (alfuzosin,doxazosin, tamsulosin, terazosin) are used to treat benign prostatic hyperplasia, but they possess various side effects like impotence, decreased libido, ejaculation disorder, gynaecomastia, dizziness, upper respiratory tract infection, headache, fatigue and chest pain (Patel and Chapple, 2008). Along with conventional therapy, some alternative therapies are also available to treat prostatic hyperplasia. Various *in vitro* studies have reported that fatty acids inhibit the enzymes activity (Liang and Liao, 1992). Coconut oil (Arruzabala et al., 2006), lauric and myristic acid (Veeresh Babu et al., 2010) have proven to be effective benign prostatic hyperplasia mainly due to 5 α-reductase inhibitory activities, which is due to their high content of lauric acid and myristic acid (mainly lauric).

However, there is no evidence for efficacy combination of lauric and myristic acid on testosterone induced benign prostatic hyperplasia whether oral dose with combination of lauric/myristic acid could prevent testosterone induced hyperplasia in rats.

MATERIALS AND METHODS

Animals

Male Wister rats weighing 180 to 220 g were procured from an institutional animal facility centre. They were housed individually in clean and transparent polypropylene cages maintained at room temperature with 12 h light/dark cycle and had free access to food and water. After 7 days of acclimatization, they were randomly distributed into experimental groups. All the experimental procedure

was carried out in accordance with Committee for the Purpose of Control and Supervision of Experimental on Animal (CPCSEA) guidelines.

Chemicals

Lauric acid and myristic acid (obtained from Sigma-Aldrich Pvt. Ltd). Finasteride (was obtained from FINAST, Dr.Reddy's Lab). Testosterone Propionate (was Courtesy of Genesis pharmaceuticals, japan). ELISA Kit for measurement of testosterone, hydrotestosterone, and Alanine transaminase (ALT), aspartate transaminase (AST) Kits were (purchased from Transasia Bio chemical Ltd Daman).

Experimental BPH model and drug administration

Lauric acid and myristic acid were suspended in distilled water using Tween 80 and administer orally. Testosterone propionate was diluted with distilled water using Tween 80 and injected subcutaneously. Experimental groups were divided into 5 groups. Group I (normal control group) did not receive any treatment. Testosterone treated groups were randomly divided into four groups (n = 6): Group II (positive control) testosterone propionate (TP, 3 mg/kg body weight); Group III, which received finasteride (10 mg/kg body weight) administered orally and TP (3 mg/kg body weight), Group IV: Lauric acid and myristic acid (180 and 70 mg/kg) administered orally and TP (3 mg/kg body weight) injected subcutaneously. Group V: Lauric + myristic acid (360 and 140 mg/kg) administered orally and TP (3 mg/kg body weight). All rats were treated once a day for four weeks.

Body and prostate weight – ratio of prostate weight to body weight and percentage of inhibition

Animal were sacrificed after weighing by sodium pentobarbital and prostates were removed and weighted immediately.
Then, prostate weight to body weight ratio were calculated. Further percentage of inhibition was calculated as follows:

100-[(treated group - negative control) / (positive control - negative control × 100]

Blood and tissue samples

After a treatment period of 4 weeks and an overnight fast, the rats were anesthetized with sodium pentobarbital (100 mg/kg body weight, i.p.). Blood samples were collected and centrifuged at 2000 × g for 10 min. The prostate was collected from each rat and weighed. All prostatic specimens from each group were fixed with 10% buffered formalin for 24 h.

Measurement of DHT and testosterone in prostate and serum

The prostate tissue was homogenized in lysis buffer containing protease inhibitors (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.1% SDS, 1 mM EGTA, 100 µg/ml PMSF, 10 µg/ml pepstatin A, and 100 µM Na_3VO_3). The homogenates were centrifuged at 12,000 × g for 25 min at 4°C, and the protein concentrations in the supernatant fractions were determined using Bradford reagent. DHT and testosterone levels in the serum and

Table 1. Effect of combination of lauric and myristic acids on prostate enlargement in testosterone treated rats.

S/N	Group	PW (g)	% inhibition	PW/BW ratio (× 10^{-3})	% inhibition
1	Group I	1.55±0.10	-	2.555±0.01358	-
2	Group II	3.65±0.55	-	3.987±0.0845	-
3	Group III	1.98±0.28*	79.71	2.016±0.01096*	82.11
4	Group IV	2.40±0.26*	59.93	2.068±0.005587*	62.12
5	Group V	2.10±0.24*	73.93	2.256±0.00975*	78.69

PW: Prostate weight, BW: body weight, Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg). Values are expressed as mean± S.E.M. Statistical analysis is done by one way ANOVA followed Dunnett's multiple comparisons test.

Table 2. Effect of combination of lauric and myristic acids on serum testosterone and DHT level.

S/N	Group	Dose (mg/kg)	Testosterone (pg/ml)	DHT (ng/ml)
1	Group I	-	134.07±20.1	8.97±1.20
2	Group II	-	207.04±39.2	19.23±3.46
3	Group III	10	170.02±28.3	14.36±2.36
4	Group IV	180 and 70	175.23±26.2	12.23±1.23
5	Group V	360 and 140	169.23±23.2	10.56±2.36*

Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg). Values are expressed as mean± S.E.M. Statistical analysis is done by one way ANOVA followed by Dunnett's multiple comparisons test.

prostates were measured with ELISA kits according to manufacturer's instructions (Transasia Bio chemical Ltd Daman).

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

ALT and AST levels were determined to assess liver function using commercial kits (Transasia Bio chemical Ltd Daman) and an auto analyzer

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis is done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test.

RESULTS

Evaluation of prostate enlargement

Prostate weights

Significant enlargement of prostate weights was found by testosterone treatment when compared with negative control. But significant reduction in elevation of prostate weights was found in group-V in testosterone treated rats. Percentage inhibition was 73.93% (Table 1).

#### *Prostate weights to body weight ratio*

In Group II, significant elevation in prostate weights/body weight ratio was noted when compared with Group I rats. But significant reduction in elevation of prostate weight/body weight ratio was observed by Group IV and V. Percentage inhibition was 62.12 and 78.69%, respectively when compared with Group I (Table 1).

#### *Measurement of DHT and testosterone in serum*

There was no significant difference in testosterone levels in serum found between all groups except for the group II (Table 2). DHT levels in serum, in group II, increased statistically more than Group I (8.79 ± 1.20 ng/ml). Conversely, in Group III (finasteride) (14.45 ± 2.36 ng/ml) markedly decreased the DHT levels in serum compared with the Group II. Group IV (10.56 ± 2.36 ng/ml) also significantly decreased the DHT levels in serum as well as the result of finasteride group (Table 2).

Measurement of DHT in prostate

As shown in Graph 1, the DHT level of group II (20.4 ± 7.87 ng/ml) significantly increased more than the normal

Graph of DHT level
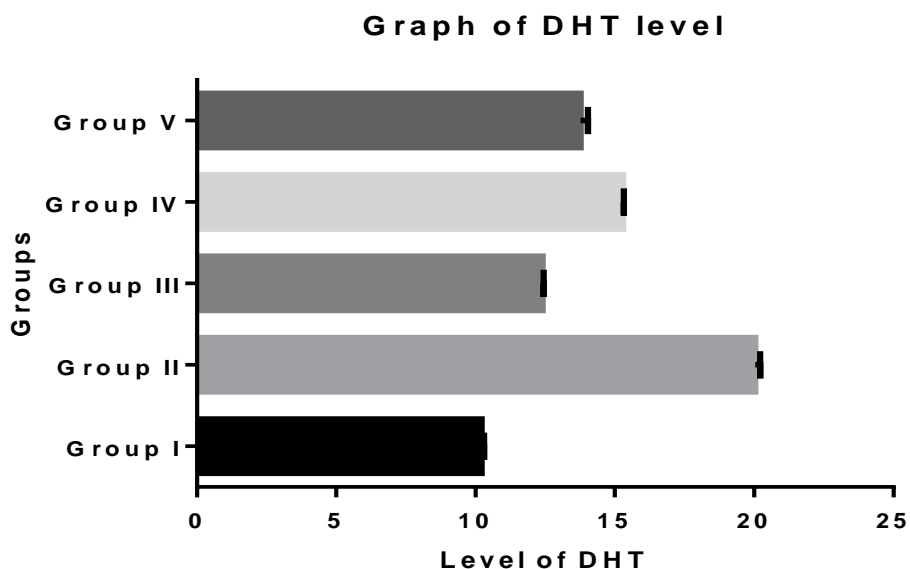
Graph 1. Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg).

Graph of Testosterone Level
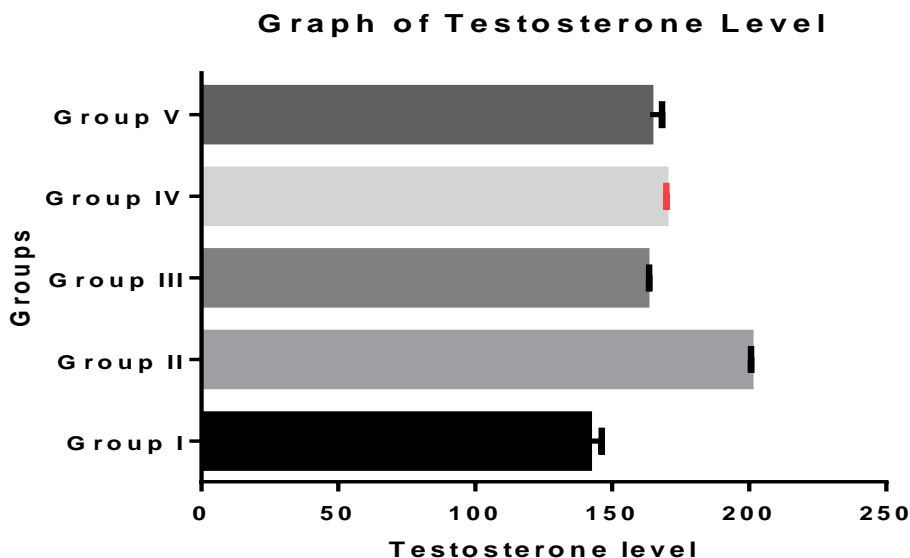
Graph 2. Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg).

control (Group I) (10.4 ± 0.60 ng/ml). In contrast, the finasteride group (11.36 ± 6.77 ng/ml) significantly decreased the DHT level in prostate compared with the Group II. The group V (14.25 ± 9.70 ng/ml) also observed the significant reduction in the DHT level of prostate in comparison with Group II, which was similar to the result of finasteride group.

Measurement of testosterone in prostate

As shown in Graph 2, the testosterone level of group II (209.04 ± 6.87 pg/ml) significantly increased more than the normal control (Group I) (135.07 ± 0.50 pg/ml). In contrast, the finasteride Group III (172.02 ± 3.77 pg/ml) significantly increased the testosterone level in prostate
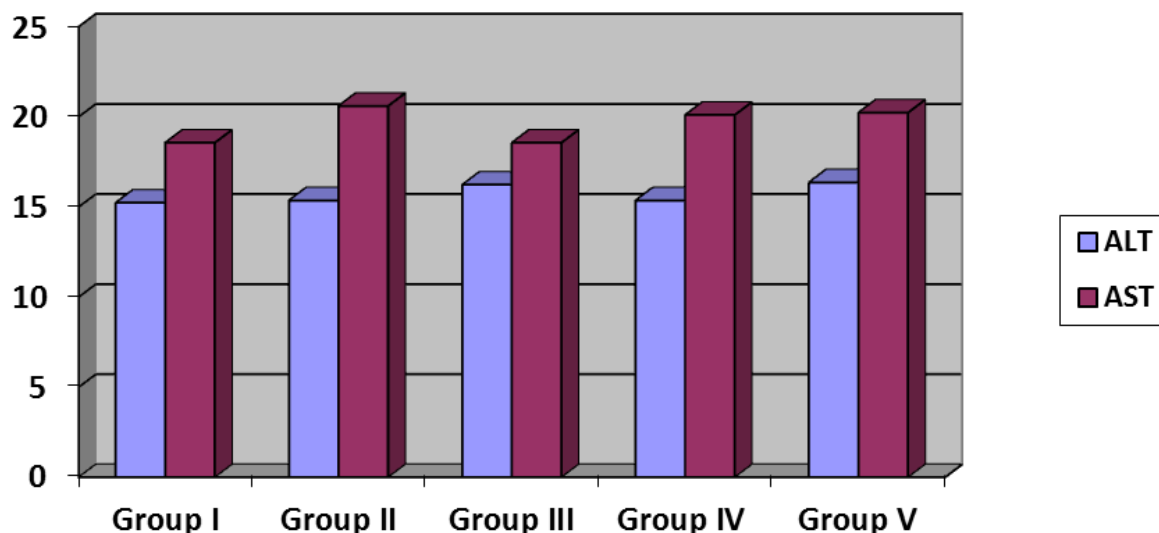
Figure 1. Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg).

compared with the Group I. The group V (174.23 ± 8.70 pg/ml) also observed the significant reduction in the testosterone level of prostate in comparison with the Group II, which was similar to the result of finasteride Group III.

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

Treatments did not affect the activities of the serum toxicity marker enzymes, ALT and AST, indicating normal liver function (Figure 1).

DISCUSSION

The present study examined the effects of combination of lauric and myristic acid in a testosterone induced rat model of BPH and compared them with the effects of finasteride, which is currently used to treat BPH. Treatment with lauric and myristic acid for 4 weeks significantly inhibited the development of testosterone induced prostatic hyperplasia, which was evident in the reduction in the elevated prostate weight/body weight ratio, reduced DHT levels in the prostate and serum.

The increased prostate weight is used as one of crucial markers of BPH according to previous study (Pais, 2010). BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement. In previous studies, animals with BPH had a significant increase in prostate weight compared with normal control animals, whereas those of animals treated with

finasteride or others herbal remedies for the management of BPH had meaningfully reduced compared with BPH animals (Bisson et al., 2007; Pais, 2010). For these reason, many studies have evaluated the inhibitory effects of various materials on the development of BPH by measuring prostate weight (Jang et al., 2010; Veeresh Babu et al., 2010). In the present study, the animals with BPH showed significantly increased prostate weight compared with the normal control animals; however, combination of lauric and myristic acid animals showed the significant reduction in these measures compared with the BPH animals.

The two main classes of drugs used as BPH treatments are inhibitors of α1-adrenoceptor inhibitors, which inhibit smooth muscle cell contraction (Furuya et al., 1982), and inhibitors of Type II 5 α -reductase, an enzyme responsible for the conversion of testosterone to the more potent androgen dihydrotestosterone (DHT) (Griffiths and Denis, 2000). Steroid 5 α -reductase converts testosterone to DHT, an active form of androgen, in the prostate. It has been noticed in earlier study that increased production of DHT results in the development of prostatic hyperplasia (Pais, 2010). Because DHT has a 10 times higher affinity for the androgen receptor than testosterone, DHT easily binds to androgen receptor, which stimulates the transcription of growth factors that are mitogenic for the epithelial and stromal cells for prostate (Carson and Rittmaster, 2003). The importance of DHT in prostatic hyperplasia was demonstrated by previous studies in which an inhibitor of 5α-reductase was administered to experimental animals with BPH (Roehrborn, 2011). For instance, at clinical doses, finasteride is selective for 5 α-reductase and

achieves about 70% suppression of serum DHT and 68 to 86% suppression of intraprostatic DHT (Span et al., 1999). These findings are in agreement with results of present study. Moreover in this study, combination of lauric and myristic acid reduced the DHT levels in the prostate and serum relative to those in rats with testosterone-induced BPH. Thus, these results indicate that combination of lauric and myristic acid inhibits the development of BPH via down-regulation of DHT.

Interestingly, although testosterone level in finasteride-treated group was observed no significant difference, it was decreased compared with the BPH animals. Before performing the present study, it was expected to have an increase in testosterone level as observed in previous studies (Pais, 2010; Roehrborn, 2011). However, this finding is different from previous studies and unexpected. Many researchers have conducted the studies on relationship between drug treatment and testosterone level in BPH condition. In most previous studies, administration of finasteride showed that the testosterone level is increased compared with that of BPH animals in many studies due to inhibition of the transformation testosterone to DHT. On the other hand, some previous studies showed that the testosterone level is similar to that of BPH animals following drug treatment as well as the result of the present study (Gasco et al., 2007). However, we did not find the clear reason why testosterone level is not changed in present study.

Conclusion

Based on the results of present study, it is concluded that oral administration of combination lauric acid and myristic acid in a BPH rat model significantly decreased the prostate weight, prostatic epithelial hyperplasia, and DHT levels in the serum and prostate. These results indicate that combination of lauric acid and myristic acid may effectively inhibit the development of BPH and may be useful for treatment of BPH patients. However further study is required to explore the effects these to fatty acids on 5 α-reductase inhibitor activities and underlying major molecular mechanisms of their action.

Conflict of Interests

The authors have not declared any conflict of interests.

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