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Preliminary study on the altered expression of 3β HSD gene in rat testis after Amlodipine and its modification by *Astercantha longifolia* seed extract

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ABSTRACT

Plan: *To verify the effect of Amlodipine on 3 β HSD gene expression in rat testis and its modification upon stopping the drug / co administration of Astercantha longifolia seed extract.*
Preface*: Though a few data are available on the potential of Amlodipine to adversely affect and that of Astercantha longifolia seeds to potentiate the male reproductive parameters, no studies are available on the effect of these on gene expression.*
Methodology*: Different groups of rat were treated orally with Amlodipine and Amlodipine plus Astercantha longifolia seed extract. After treatment the Amlodipine treated group was kept untreated for recovery. Finally total RNA from all groups were extracted and PCR for 3 β HSD gene was performed and products were analyzed by gel electrophoresis.*
Outcome*: Amlodipine treated groups showed down regulation of 3 β HSD expression but the same could be reversed after the recovery period of about 67 days. In groups treated with Amlodipine and Astercantha longifolia seed extract, 3 β HSD expression was up regulated in a dose dependent manner. It may be concluded that as 3 β HSD is involved in the conversion of cholesterol to testosterone, the decrease / increase in testosterone level may be due to the down / up regulation of 3 β HSD expression .*
Key words- *Amlodipine, Astercantha longifolia, 3 β HSD gene*

1. INTRODUCTION

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse. Compared to the past infertility among married couple is a chronic problem now-a-days. Studies show that in our state 20 % of couples suffer some type of infertility due to multiple reasons. Male factor contribute to 45% of these infertilities[1]. Calcium channel blockers (CCB) are a widely used class of drugs for their reliable antihypertensive, antiarrhythmic or antianginal effects.

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But many data accumulated over the past few years have revealed that these agents have the ability to cause infertility in males.[2] In a study to determine the effect of the widely used calcium channel blocker Amlodipine on serum testosterone, testicular weight and gonado somatic index (GSI), it was found that Amlodipine is capable of causing a significant reduction in serum testosterone, testicular weight and GSI when used in a dose of 0.14 mg/kg per day for 50 days[3]. Similarly another study reported a 23 % reduction in sperm count when Amlodipine was used in a dose of 0. 4 mg/rat per day for 30 days.[4]

In our earlier studies[5] with the clinically equivalent dose of Amlodipine (10mg/day) in albino rats in their reproductive age for different periods ranging from two weeks to eighteen weeks, the reproductive parameters such as sperm count, motility and GSI were found to be adversely affected in a significant and duration dependent manner pointing to potential risk of this drug on male fertility.

Though the adverse effects of Amlodipine on sperm parameters are confirmed no reports are available on its effect on gene expression. 3β-HSD is one of the key enzymes in the biosynthesis of androgens and almost all other biologically active steroids. High 3β-HSD activity in the testes is essential for normal steroidogenesis and subsequently for reproduction. Clearly, expression of 3β-HSD in the adult animal is an indicator of testicular androgen production[6]. Similarly the expression and activity of 3β-HSD depend on the presence of several endogenous as well as exogenous toxic compounds[7]. So in the present study we plan to determine the effect of Amlodipine on the expression of 3beta HSD gene in rat testis.

Besides, *Asteracantha longifolia* is a plant common in India and traditionally its seeds are used for the treatment of sexual debility, premature ejaculation, erectile failure and oligospermia[8,9] After confirming the absence of any toxic effect for the ethanolic extract of *Asteracantha longifolia* seeds through acute and sub acute toxic study,[10] we intend to determine the effect of two different doses of *Asteracantha longifolia* seed extract on Amlodipine induced change in the expression of 3beta HSD gene in rat testis if any through RT-PCR (reverse transcription-polymerase chain reaction) which is a sensitive method for the detection of mRNA expression levels. Reverse transcription (RT) followed by a polymerase chain reaction (PCR) represents the most powerful technology to amplify and detect trace amounts of mRNA[11]. The Housekeeping genes β actin which is a cytoskeleton protein is used as the internal control here to indicate perfect nucleic acid extraction and quality of samples[12].

Thus the present study aims to determine

1. The effect of Long term use of Amlodipine in clinically equivalent dose on the expression of 3 β HSD gene in the testis of Wistar albino rats.
2. To verify the reversal of gene expression changes if any, upon stopping the administration of Amlodipine.
3. Modification of (1) by the co administration of ethanolic extract of *Astercantha longifolia* seeds.

2. MATERIALS AND METHODS

Ethical clearance: The study protocol was approved by the Institutional Animal Ethical Committee, Medical College, Thiruvananthapuram.(IAEC No 02/15/2010/MCT dated 08-06-2010). Ethanolic extract of the seeds of *Asteracantha longifolia* was used for the present study.

Healthy male albino rats of 5-6 months of age (Wistar strain) weighing 180-220 gm procured from the animal house of Medical College, Thiruvananthapuram were used for the study, These animals were fed on standard pellet diet (manufactured by Nav Maharashtra Chakan Oil Mills Ltd; Pune and supplied by Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. The animals were maintained under standard conditions of relative humidity, 12 hrs light-dark cycle, adequate ventilation and ambient room temperature.

These rats were divided into different groups and given treatment such as

Group I- control- Distilled water in a dose of 1ml/100gm body weight.
Group II- Treated with Amlodipine 0.9 mg/kg p.o once daily for 65 days
Group III- Treated with Amlodipine 0.9 mg/kg p.o once daily followed by kept untreated for 67 days
Group IV- Treated with Amlodipine 0.9 mg/kg p.o once daily and Asteracantha longifolia seeds extract 100 mg/kg p.o for 65 days
Group V- Treated with Amlodipine 0.9 mg/kg p.o once daily and Asteracantha longifolia seeds extract 250 mg/kg for 65 days
Group VI- Treated with Amlodipine 0.9 mg/kg p.o once daily for 107 days

At the end of the specified period, animals were weighed and killed by cervical dislocation, the peritoneal cavity was opened through a lower transverse abdominal incision and the testicles were removed for RNA isolation.

Semi quantitative reverse transcriptase-polymerase chain reaction[13]

Total RNA from treated animals were extracted using TRIzol reagent as per manufacturers instruction (Invitrogen). Purified RNA was used for reverse transcription and amplification using MMLV one step RT PCR kit (Merck Genei). PCR was performed using the following primers

5'TGAAAAATGGTGGCACACTGC 3' (Forward) and
5' TATAGTTGTAAAATGGACGCAGC 3' (Reverse),
ß actin 5' GAGACCTTCAACACCCCAGC 3' (Forward) and
5' CACAGAGTACTTGCGCTCAG-3', which was used as the house keeping gene.

Amplification was performed using a thermal cycler (Eppendorf Master Cycler, Germany) as per the following cycles. c DNA synthesis was performed at 42^{o}C for 30 minutes followed by inactivation at 94 ^{o}C for 15 minutes. 30 cycles of amplification were performed with denaturation at 94 ^{o}C for 1 minute, annealing at 60 ^{o}C for 1 minute and extension at 72 ^{o}C for 1 minute 30 seconds. A final extension was performed for 5 minutes at 72 ^{o}C.

The PCR products were run on a 1.5% agarose gel containing ethidium bromide. Band intensity was normalized to values for ß-Actin that was used as the internal control using Image J analysis software and expressed as arbitrary units.

3. RESULTS AND DISCUSSION

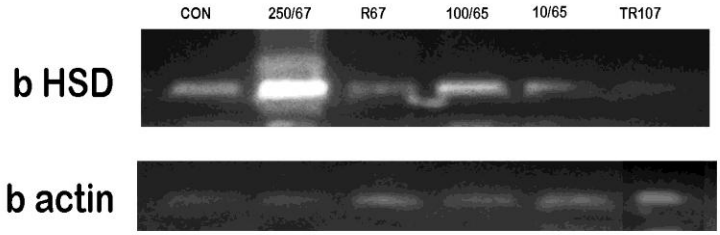

Fig. 1: Expression analysis of β HSD in treated groups

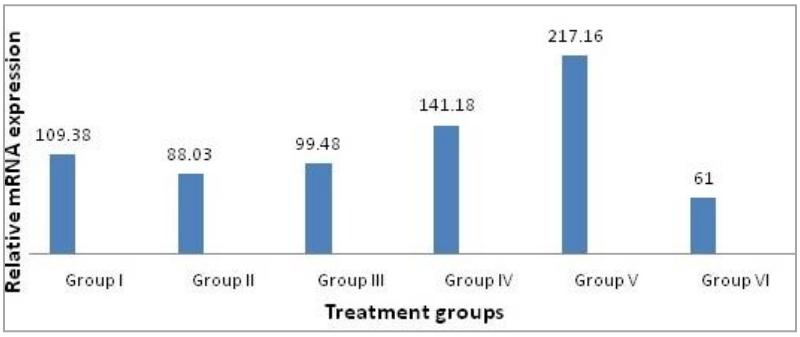

Fig II-3β HSD expression in different groups

Group I-Untreated control
Group-II-Amlodipine equivalent to 10 mg /day for 65 days
Group-III-Amlodipine equivalent to 10 mg /day for 65 days and kept untreated for 67 days
Group-IV-Amlodipine equivalent to 10 mg /day and extract {100mg/kg} for 65 days
Group-V-Amlodipine equivalent to 10 mg /day and extract {250mg/kg} for 65 days
Group-VI-Amlodipine equivalent to 10 mg /day for 107 days

From the figures it can be clearly observed that the oral administration Amlodipine to Wistar albino rats in a dose equivalent to 10mg/day (0.9mg/kg) for 65 days (Group II) has produced 19.5% supression of 3β HSD expression when compared to the control (Group I) but the same could be reversed almost to the pretreatment level after 67 days of recovery period. (Group III) (Only 9% of suppression). At the same time the co administration of *Astercantha longifolia* seed extract along with Amlodipine in a dose of 100mg/kg and 250mg/kg for 65 days has produced 29% (Group IV) and 98.5% (Group V) increase in 3β HSD expression when compared to the control. But the administration Amlodipine alone for a long period of 107 days (Group VI) has produced 44.2% supression of 3β HSD expression.

4. CONCLUSION

Calcium channel blockers (CCB) are a widely used class of drugs for their reliable antihypertensive, antiarrhythmic and antianginal effects. Clinical reports on the suspected role of Nifedipine, a widely used calcium channel blockers to cause infertility in healthy men were made in 1994 and subsequently some animal studies were also made in this aspect. Amlodipine, the widely used calcium channel blocker having good bioavailability also possess the potential to adversely affect the reproductive parameters in male albino rats even when used in low clinically equivalent doses. But the actual reason for this adverse effect could not be clearly traced out yet. Here, in this pilot study to verify the effect on gene expression it is seen that Amlodipine causes considerable decrease in the expression of 3β HSD gene which is involved in the conversion of cholesterol to testosterone. Optimum testosterone levels are necessary for optimum sexual function.Reduced formation of testosterone which is the key hormone for male sexuality may be the reason for Amlodipine induced adverse effects on testicular parameters. At the same time return of gene expression level almost near to the control values upon stopping the treatment for 67 days indicate that the Amlodipine induced testicular impairment can be reversed without any intervention

Moreover the dose dependent increase in the gene expression by the co administration of ethanolic extract of *Astercantha longifolia* gives scientific support for its traditional use as a male fertility booster. This herbal preparation may be taken along with Amlodipine especially by men in their reproductive age for countering the ill effects of the latter on reproduction. More detailed studies are required to ascertain the pattern of inhibition of Amlodipine on gene expression of 3β HSD gene.

Besides it is well learned that voltage-gated calcium channels play a role in excitation-secretion coupling, neurotransmitter release and regulation of gene expression. In steroid producing cells, the steroid hormone biosynthesis is calcium dependent and intracellular calcium rise is essential for maximal stimulation of steroidogenesis. When Amlodipine blocks the voltage gated calcium channels, Astercantha longifolia seed extract may be enhancing the intracellular calcium levels. But the *Astercantha longifolia* seed extract employed in this study is a crude extract containing a mixture of different constituents. So further attempts are needed to find out whether the dose dependent increase in the gene expression by the co administration of ethanolic extract of *Astercantha longifolia* along with Amlodipine is due to any stimulant effect of this compound on intracellular calcium release / calcium entry through voltage gated calcium channels.

Detailed investigations are warranted to find out whether the co administration of *Asteracantha longifolia* seed extract produces any antagonistic effects on the antihypertensive potential of Amlodipine.

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