

Available online on 15.09.2018 at <http://jddtonline.info>

## Journal of Drug Delivery and Therapeutics

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Research Article

# EFFECT OF METOSARTAN ON MALE REPRODUCTIVE POTENTIAL IN NORMAL AND HYPERTENSION INDUCED RATS

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## ABSTRACT

Hypertension is one of the long term medical problems in world leading to mortality of 18% globally. Epigenetic modifications like gene regulation, DNA histone modification and methylation patterns are mostly affected during Hypertension condition and need to be carefully monitored. So, metosartan is the one of the antihypertensive drug used to study its effects on genome especially in *In-vivo* condition. Metosartan doesn't affect the chromatin integrity in hypertensive rats but reduces the sperm count drastically and reaches up to zero at 1.25mg/ml concentration. Majorly the drug affects the sperm count but not the maturation of sperm. In hypertension condition the sperms are aggregated in to complex where as in metosartan treated group *In-vitro* lead to severe looping of sperms and resulted in formation of apoptotic bodies. In normal rats excessive DNA fragmentation is seen in metosartan treated group and treatment with RNase A along with metosartan *in-vitro* restored the chromatin integrity but failed to give promissive results in *In-vivo* condition. So, study of genome integrity in hypertensive patients treated with metosartan is compulsory as it affects chromatin integrity adversely in *In-vitro* condition in normal rats.

Keywords: Hypertension, Genome integrity, Azoospermia, Teratozoospermia, Metosartan

**Article Info:** Received 08 Aug, 2018; Review Completed 01 Sep 2018; Accepted 02 Sep 2018; Available online 15 Sep 2018

### Cite this article as:

Beeram E, Effect of metosartan on male reproductive potential in normal and hypertension induced rats, Journal of Drug Delivery and Therapeutics. 2018; 8(5):400-406

DOI: <http://dx.doi.org/10.22270/jddt.v8i5.1891>

## INTRODUCTION

Metosartan is normally used to treat hypertension but its effects on reproductive potential were not up to the mark. Angiotensin-II is the effector molecule that controls hypertension by acting via two types of receptors namely angiotensin receptor type I and angiotensin receptor type II. Angiotensin receptor type I is expressed ubiquitously where as angiotensin type II expresses only in certain tissues. Metosartan mainly blocks the beta receptors on the heart and also angiotensin type I receptor. *In-vitro* results of metosartan have confirmed the deleterious effects of metosartan on the testes and sperms. So, in vivo confirmation of results is necessary for the effective and safe medication of the drug. Normal sperm count ranges from 15 million to 200 million sperms per ml of ejaculate. Drugs like antidepressants, alpha adrenergic blockers and calcium

channel blockers affect the sperm quality and sperm count<sup>1</sup>.

Metosartan is also one of the drugs that effects sperm count drastically in male wistar rats. However in human subjects it needs to be tested. Chromatin integrity is the main factor that is affected by drug metosartan in testes and sperms in invitro. Treatment with drug leads to apoptosis in rats by cyt C release from mitochondria<sup>2</sup>. RNase A is the ribonuclease secreted by the pancreas and involved in digestion of RNA present in the food. It is very difficult to inactivate RNase A, so all the experiments are carried out at room temperature.

RNase A treatment lead to membrane dissolution and recondensation of chromatin in *In-vitro*<sup>3</sup>. Recently a ribonuclear protein complex containing Rae 1 is proved to be necessary for mitotic spindle assembly. So, RNA

is not only part of the chromatin but also necessary for the activity of Rae 1<sup>4</sup>. DNA fragmentation was profound in sperm than testes associated with metosartan treatment in hypertension induced rats.

Azoospermia is the condition seen in hypertension induced rats kept under medication with metosartan at concentration greater than 1mg. Mitochondrial viability was unaffected with metosartan in rats compared to other parameters. But with RNaseA the mitochondrial viability was less and resulted in constant functioning in *In-vivo* at low concentrations. In B.P induced rats high sugar concentration leads to uncontrolled stress condition which may affect the mitochondria due to continuous operation of electron transport chain. This may leads to oxidative stress in these rats.

Due to high sugar concentration and intermediary metabolism of sugars to fat there is large granulation of testes as it is involved in synthesis of gonadal hormones like testosterone. Various sperm abnormalities like teratozoospermia is associated with metosartan in both *In-vitro* and *In-vivo* conditions. However RNase A protection against chromosomal stability was known recently which removal of R loops which causes instability<sup>5</sup>. Numbers of viable sperms are also found to be reduced in metosartan medicated rats *in vivo*. However the metosartan affects the reproduction potential of the male rats in adverse manner and causes apoptosis in sperm and testes leading to male infertility. The objective of the study is mainly to study the effect of drug on genome integrity and reproductive potential of male rats during hypertension condition.

## MATERIAL AND METHODS

### Experimental design:

Male wistar rats of body weight ranging between 250-300g was divided in to three groups and administered with 10% fructose in drinking water to induce hypertension and monitored for 1 week. After 1 week the blood glucose levels are measured and recorded. After confirmation of hypertension induction in the rats metosartan commercially available of potential 25mg of metoprolol and telmisartan 45mg was administered orally with concentration of 500µg/ml followed by 1mg/ml and 1.25 mg/ml in the alternate days to each animal in the group separately and one of the animal is sacrificed in the group on the next day and the experiments are performed. During the treatment period with metosartan the hypertension was maintained sustainly through 10% fructose. The animals are sacrificed after 1 day and sperm count, genome integrity assays and agarose gel electrophoresis of sperm samples were done accordingly.

### Genome integrity assays:

#### Aniline blue staining:

The protocol was followed as per cited reference<sup>14</sup> with minor modifications which are briefly explained here. Five microliters of minced a testis suspension was placed on the glass slide, smear was made, air dried and fixed in 3% glutaraldehyde solution in 0.2M PBS of pH

7.4 for 30 min. After the incubation of the slides with fixative they were stained with 5% acidic aniline blue stain (5g aniline blue in 100ml of 4% glacial acetic acid in double distilled water, pH 3.5) for 5 min, air dried and observed under the compound microscope at 10X x 20.

### Comet assay:

Standard comet assay as per cited reference<sup>15</sup> 100µl of the isolated mitochondria pellet was mixed with 1000µl of 0.8% agarose and 100µl of mixed suspension was spread on the slide and kept in ice for 45min. After casting, the slides were immersed in lysis buffer (2.5% SDS in 45 mM Tris-borate, 1 mM EDTA, pH 8.4) for 20min and incubated in electrophoresis buffer for 5min. Electrophoresis was carried out with 45mM TBE buffer with pH 8.4 at 2v/cm for 2min and Ethidium bromide staining was performed and visualised using fluorescent microscope.

### Sperm count:

Epididymis was dissected using sterile scissors and the sperms were collected by making 5 to 6 incisions on the epididymis up to the liquid becomes pale yellow and some portion of the liquid was transferred to neubar chamber which was previously adjusted using microscope and allowed to settle for 5min. sperms in the four chambers were counted and the sperm count was calculated.

### Isolation of DNA:

The protocol was as per cited reference<sup>16</sup>. Testes were homogenized in lysis buffer (50 mM Tris-Cl, pH 8.0, 100 mM EDTA, 0.125% SDS) using mortar and pestle and further 1ml of the lysis buffer was added to the collected supernatant and incubated for overnight at 55°C. To the lysate an ml of phenol: chloroform: isoamyl alcohol (25:24:1) mixture was added and centrifuged at 2000xg for 10min. Aqueous phase was collected and washed once again with 1ml of phenol: chloroform: isoamyl alcohol mixture and 3ml of chloroform. Aqueous phase was separated and DNA was precipitated with cold ethanol. Precipitated DNA was pelleted, collected and stored at -20°C.

### Agarose gel electrophoresis:

1% agarose gel was prepared and the isolated sperm DNA was loaded in to respective wells along with loading buffer and the gel was run with 1X TBE buffer at standard conditions and examined under transilluminator after the run.

### Measurement of Glucose levels in blood:

Blood glucose levels are measured by Glucometer and recorded in both test groups and control.

## RESULTS

*In-vitro* effects of drug metosartan and RNase A on testes and sperm:

From the figure 1 Control appears normal where as RNase A treatment resulted in aggregation and acrosome reacted sperms where as with both RNase A

and drug treated samples sperms are not visible in short term changes. From figure 1C and 1G Drug treated and M+R treated sample with short term changes is associated with similar consistency of chromatin as that of control. From figure 1D the chromosomes showed

abnormal synapsis of X-chr<sup>6</sup>. Drug treated sample consists of mixture of both immature and mature sperms where as in drug+ RNase A treated sample consists of more number of immature sperms.

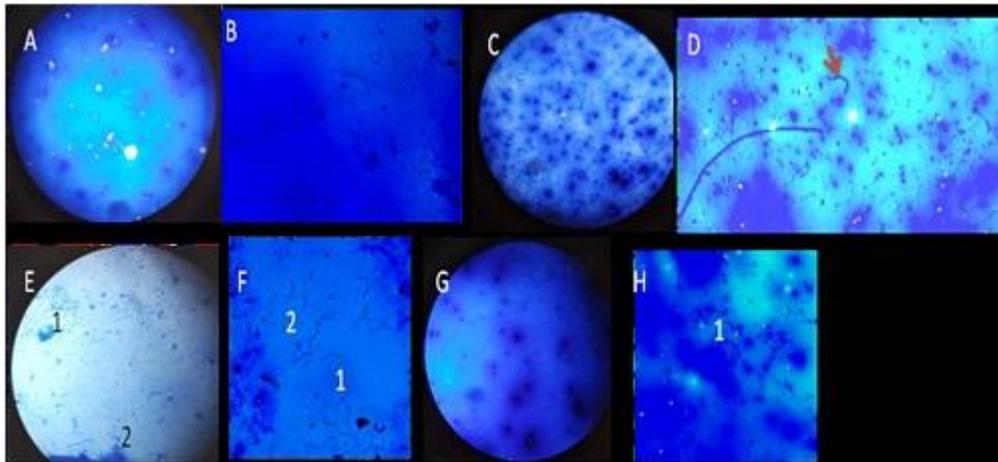


Figure 1: Short term and long term changes associated with sperm samples treated with metosartan and RNase A *In-vitro*. (A), (B) are control of short term changes and long term changes (C), (D) are short term changes and long term changes associated with Drug treated sample (E), (F) are short term and long term changes associated with RNase A treated sample (G), (H) are short term and Long term changes associated with both drug and RNase A treated sample In (E) 1 indicates aggregation of sperms and 2 indicates acrosome reacted sperm. In (F) 2 indicates abnormal sperm and 1 indicates looped sperm. In (H) 1 indicates interlinked sperms. In 1D the arrow mark shows abnormal synapsis of X chromosome.

From figure 2B control appeared normal with nucleosome the basic unit of chromatin and drug treated sample with apoptotic bodies and teratozoospermia, where as figure 2F showed condensed chromosomes which explains the involvement of Rae 1 containing ribonucleoprotein in spindle assembly which requires RNA for its activity<sup>4</sup>. During the time gap between the study of short term changes and long term changes

associated with sample the chromatin condensed in to chromosomes but consisting of abnormally separated sister chromatids. Whereas in figure 2H the RNase A + drug treated group showed reorganisation of chromatin as drug binds and inhibits RNase A so, the enzyme is not released and drug induced effects are also minimal. In 2D the drug treated sample showed severe looping of sperms and resulted in formation of apoptotic bodies.

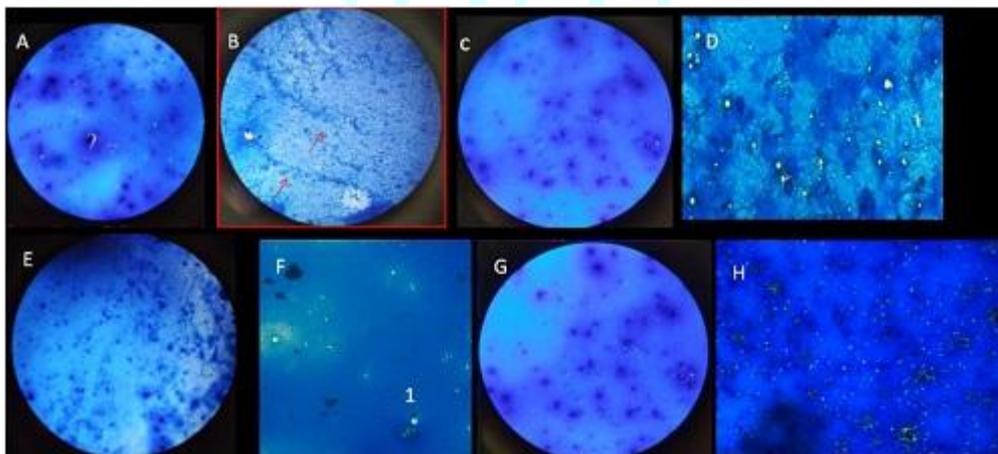


Figure 2: Short term and Long term changes in testes treated with drug and RNase A *in vitro*. (A) Short term control (B) long term control showing chromatin arranged as nucleosomes which is indicated by arrows. (C) Short term changes associated with drug treated sample (D) long term changes associated with drug treated sample (E) Short term changes associated with RNase A treated sample. (F) Long term changes associated with RNase A treated sample. (G) Short term changes associated with Drug + RNase A treated group (H) long term changes associated with Drug + RNase A treated group which showed reappearance of chromatin network. In 1(G) the Drug + RNase A and 1 (C) the drug treated samples showed similar consistency with control where as in 1(E) RNase A treatment lead to cleavage of chromatin.

### ***In-vivo* effects of drug metosartan and RNase A on testes of normal and hypertension induced rats:**

In figure 3 (A) 1 indicates chromatin basic unit nucleosome. Epigenetic mechanisms like histone modification and DNA methylation in CpG islands and involvement of miRNAs are known to be occur during B.P <sup>7</sup> and miRNA 425 correlates positively with B.P clearly indicates the role of noncoding RNAs in gene

regulation pathways during hypertension<sup>8</sup>. From figure 3 Hypertension induced control showed normal appearance of chromatin but higher order structures are not found. In *in-vivo* condition drug treated sample showed extensive DNA fragmentation where as 1E shows normal sperm structure but with immature sperms and less DNA fragmentation. 1D, 1F and 1G showed normal chromatin except in 1mg treated rats.

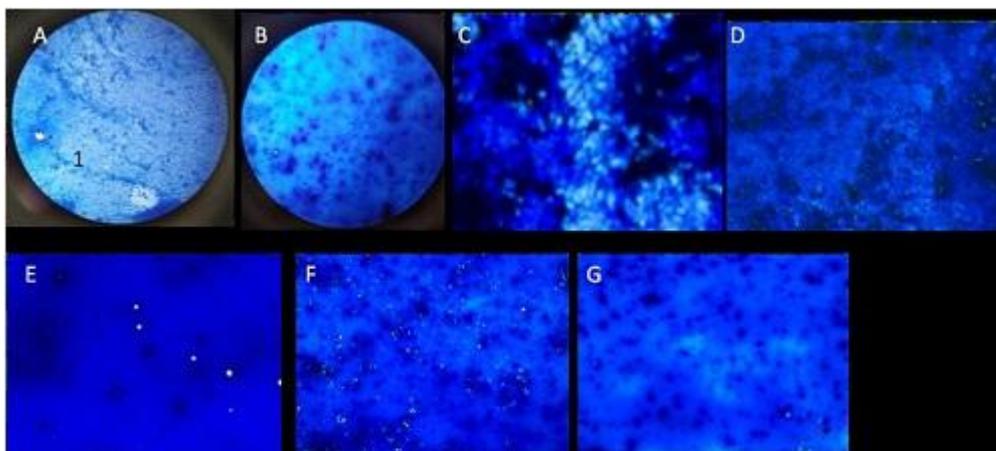


Figure 3: *In-vivo* effects of drug metosartan and RNase A in normal and hypertension induced rats treated with metosartan. (A) Control of testes of normal rats (B) control of testes in hypertension induced rats. (C) *In-vivo* drug treated rats at concentration 100µg in normal rats (D) *In-vivo* drug treated sample at concentration 1.25mg of metosartan in hypertension induced rats (E) *In-vivo* RNase A+ metosartan treated sample at concentration 100 µg in normal rats. (F) *In-vivo* drug treated sample of concentration 500 µg of metosartan in hypertension induced rats. (G) *In-vivo* drug treated sample of concentration 1mg of metosartan in hypertension induced rats.

### ***In-vivo* effects of drug metosartan on sperms of hypertension induced rats:**

From figure 4B the B.P control showed aggregation of sperms in to complex and sperms treated with low doses of metosartan doesn't affected the maturation and as flagellum consists of axoneme which is made up of tubulin complex is also observed in sperm tail <sup>9</sup>. From

figure 4D Metosartan causes complement mediated activation of cell death also in addition to apoptosis which is evident by the presence of complement regulatory factors CD55, CD46, CD59, C1-INH on sperm surface <sup>10</sup>. In spite of their presence they does not protect the sperm from C-mediated injury. Treatment of rats with metosartan at concentration of 1.25mg/ml resulted in aspermatogonia.

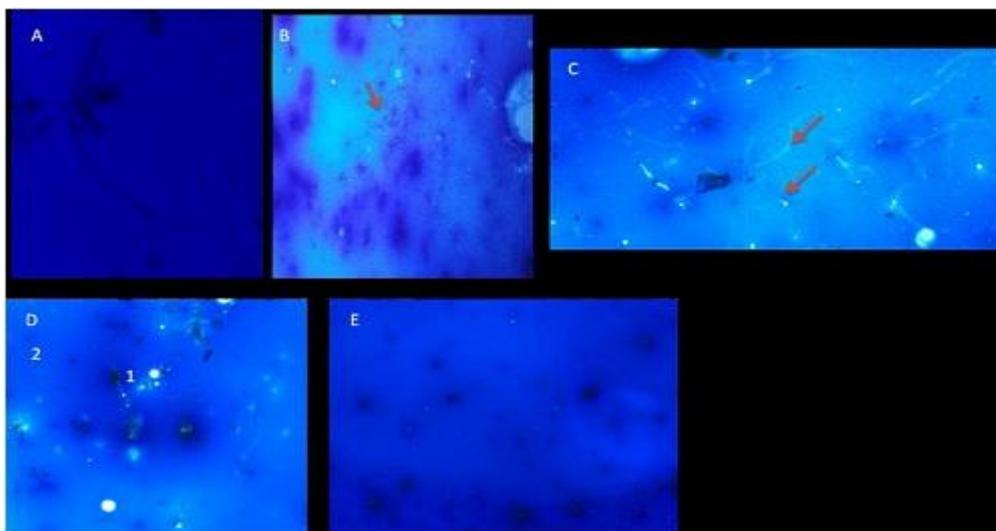


Figure 4: *In-vivo* effects of drug metosartan on sperms of hypertension induced rats. (A) control (B) Control of hypertension induced rats (C) sperms of hypertension induced rats treated with metosartan of concentration 500 µg/ml (D) sperms of hypertension induced rats treated with metosartan of concentration 1mg/ml and (E) sperms of hypertension induced rats treated with metosartan of concentration 1.25mg/ml.

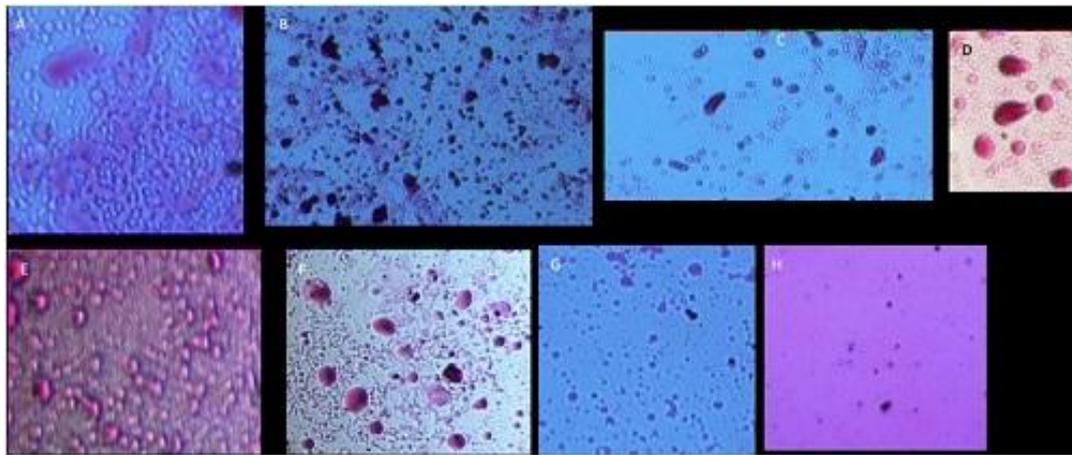


Figure 5: Assessment of genome integrity in testes of normal and hypertension induced rats by comet assay. (A) comet assay of sperm sample treated with metosartan at concentration of 500 µg/ml. comets were observed which indicates DNA fragmentation in the nucleus of the sperm. (B) Study of comets at concentration of 1mg/ml of metosartan. Most of the cells are normal and DNA fragmentation was normal compared to low dose of metosartan. (C) Examination of sperm by comet assay at concentration of 1.25mg/ml of metosartan. DNA fragmentation and ds breaks are absent at higher concentration. (D) Comet assay of testes in normal rats treated with 100 µg/100µl of metosartan. (E) Testes of hypertension induced rats at concentration 500 µg/ml. (F) Testes of hypertension induced rats at concentration of 1mg/ml of metosartan (G) Testes of hypertension induced rats with no ds breaks at concentration of 1.25 mg/ml of metosartan. (H) Comet assay of control testes.

**Comet assay of sperm and testes of normal and hypertension induced rats treated with metosartan:**

Except at low dose of metosartan in normal and hypertension induced rats all the sperm and testes samples showed normal genome integrity without any DNA fragmentation which is further supported by agarose gel run of sperm samples from figure 6. From

figure 6 S1 showed maximum DNA fragmentation compared to S2 and S3. Sperm count was increased with increase in dosage of metosartan but it resulted in aspermatogonia at higher concentrations of drug. Glucose levels are found to be normal in control but in hypertension induced rats it is drastically increased up to 427mg/dl.

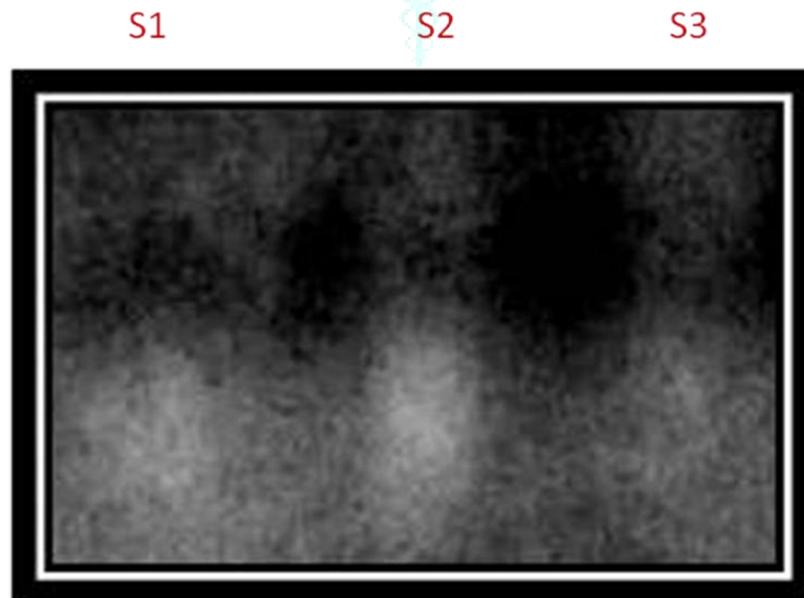


Figure 6: Agarose gel electrophoresis of *In-vivo* treated sperm samples with metosartan at 500 µg/ml (S1), 1mg/ml (S2) and 1.25mg/ml (S3). DNA fragmentation was more in S1 compared to S2 and S3.

Table 1: Semen parameters in normal and hypertension induced rats. In metosartan (1.25mg/ml) treated group aspermatogonia is observed both in testes and epididymis. In normal rats metosartan treated group was observed with highest sperm count and viability compared with metosartan+ RNase A treated group. (-) represents experiments not performed.

	Sperm count in normal rats treated with metosartan at concentration of 100 µg/100µl	No. of viable sperms in normal rats treated with 100 µg/100µl of metosartan	Sperm count in hypertension induced rats at three different concentrations
Metosartan group	11,02500sperms /ml	10,10,000 viable sperms /ml	-
Metosartan + RNase A treated group	1,20,000 sperms/ml	1,20,000 viable sperms /ml	-
Metosartan treated group of conc. 500 µg/1ml	-	-	1,0000,000 sperms/ml
Metosartan treated group of conc. 1mg/1ml	-	-	1,92,80,000 sperms/ml
Metosartan treated group of conc. 1.25mg/ml	-	-	0 sperms/ml

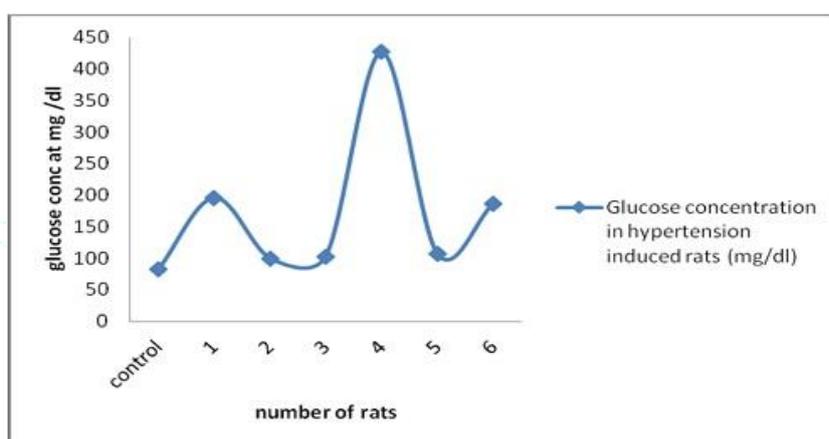


Figure 7: Glucose concentration in hypertension induced rats (mg/dl). Control consists of 82mg/dl concentration of glucose in blood where as highest glucose concentration was recorded in 5th rat with glucose concentration ranging in between 400-450 mg/dl.

## DISCUSSION

Hypertension is one of the long term medical condition characterised by high blood pressure. So, the risk of the death due to hypertension can be avoided by changing lifestyle of the person. If the hypertension cannot be controlled by lifestyle, medications that reduce blood pressure is found to be useful. Metosartan is one of such antihypertensive drug that includes two active components telmisartan and metoprolol. Telmisartan has been found to be involved in various protective effects but when given in combination as metosartan with metoprolol it resulted in deleterious effects like apoptosis, complement activation, loss of chromatin integrity. However in hypertensive rats chromatin integrity was maintained eventhough after *In-vivo* treatment with metosartan but the reproductive potential of the male rats are severely compromised at higher concentrations of drug. Sperm genome was highly fragmented even at low concentrations of drug.

Assays that detect abnormal genome integrity like comet assay showed normal results and the drug doesn't affect

maturation of sperms *in vivo*. Sperm count and sperm motility was drastically compromised in metosartan treated rats *in vivo*. In humans chr 1q is the loci associated with B.P similar to mouse and rat B.P loci associated with alterations in three genes namely ATP1B1, RGSS and SELE<sup>11</sup>. Individuals with defects in genes leads to reduction in cumulative systolic BPs ranging between 8-10mm Hg<sup>11</sup>. B.P is mainly due to defects in pathways that regulate the hypertension. However the pathways which is acted up on by metosartan include alpha and beta receptors on coronary arteries and Renin-Angiotensin system that regulates blood pressure. Other pathways related to BP is production of Hexadecanedioate by secondary fattyacid oxidative pathway<sup>12</sup>. The compound effect is related to increase in intracellular levels compared to circulating levels<sup>12</sup>.

Glucose levels, triglycerides and plasma insulin levels are mainly elevated in blood pressure<sup>13</sup> in present context it reached up to 400-450mg/dl. In normal rats treatment with metosartan leads to apoptosis through release of cytochrome C and induction of ds DNA

breaks in the genome. *In-vitro* effects are more deleterious compared to *In-vivo* results as the testicular system is directly exposed to drug and due to lack of counteracting mechanisms. In normal rats the treatment of RNase along with metosartan lead to further decrease in sperm count compared to metosartan treated group. Together the use of metosartan effects with respect to high concentration in BP patients should be monitored carefully otherwise which may leads to loss of reproductive potential in male subjects. The effects are temporary or permanent should also be kept in mind.

## CONCLUSION

Telmisartan one of the active agent in the drug metosartan induces ds DNA breaks in the genome and metoprolol known to effect semen parameters .so, it is

necessary to study the effect of two as the combined agent metosartan to know the drug- drug interactions. Metosartan effect on male reproductive potential was adverse with azoospermia and teratozoospermia in the hypertensive rats and eventhough it doesn't effect the maturation, it does it in synthesis of sperms in testes. Treatment of rats along with metosartan and RNase A resulted in restoration of chromatin integrity it failed incase of *In-vivo* condition in normal rats. Genome integrity was not affected in hypertension induced rats compared to normal rats. Together the drug effects the sperm count, sperm viability and testes viability in normal rats where as it affects sperm count especially in hypertensive rats leading to azoospermia and abnormal synapsis of X-chr in male rats induced with hypertension.

## REFERENCES

1. Paul R. Brezina , Fahd N. Yunus , Yulian Zhao., Effects of Pharmaceutical Medications on Male Fertility. *J Reprod Infertil.*; 2012,13(1):3-11.
2. Eswari Beeram and Thyagaraju Kedam ., Apoptosis of testis tissue by drug metosartan through activation of intrinsic pathway by the release of Cyt C from mitochondria *International Journal of Chemical Studies* ; 2018, 6(1): 614-617.
3. Eswari Beeram\* and Thyagaraju kedam., Chromatin integrity in testis by microscopy and drug inhibition studies of rnase a by uv visible spectroscopy. *ejbps*, 2018, 5( 01), 232-237.
4. Michael D. Blower, Maxence Nachury, Rebecca Heald, and Karsten Weis (2005)A Rae1-Containing Ribonucleoprotein Complex Is Required for Mitotic Spindle Assembly Cell, 121, 223–234.
5. Caiyun Wu, Liu Wang, Furhan Iqbal, Xiaohua Jiang, Ihtisham Bukhari, Tonghang Guo, Gengxin Yin, Howard J. Cooke, Zhenyi Cao, Hong Jiang and Qinghua Shi., Preferential Y-Y pairing and synapsis and abnormal meiotic recombination in a 47,XXY man with non obstructive azoospermia. *Molecular Cytogenetics*, 2018, 9(9), 1-9.
6. Andre´ s Aguilera and Tatiana Garcı´ a-Muse., R Loops: From Transcription Byproducts to Threats to Genome Stability *Molecular Cell*, 2012, 46, 115-122.
7. Ingrid A. Wise and Fadi J. Charchar., Epigenetic Modifications in Essential Hypertension *Int. J. Mol. Sci.*, 2016, 17, 451; doi:10.3390/ijms17040451
8. Zhou Zhang, Brian Thomas Joyce, Jacob K. Kresovich, Yinan Zheng, Jia Zhong, Ruchi Patel, Wei Zhang, Lei Liu, Chang Dou, John P. McCracken6, Anaite DõÁaz, Valeria Motta, Marco Sanchez-Guerra, Shurui Bian, Pier Alberto Bertazzi, Joel Schwartz, Andrea A. Baccarelli, Sheng Wang, Lifang Hou., Blood pressure and expression of microRNAs in whole blood. *PLOS ONE*, 2017, DOI:10.1371/journal.pone.0173550.
9. Jana Pe´knicova´, Alena Kuba´tova´, Vadym Sulimenko, Eduarda Dra´berova´, Vladimı´ r Viklicky, Pavel Hoza´k, and Pavel Dra´ber., Differential Subcellular Distribution of Tubulin Epitopes in Boar Spermatozoa: Recognition of Class III b-Tubulin Epitope in Sperm Tail Biology Of Reproduction, 2001, 65, 672–679.
10. Osmond J. D'Cruz, Gilbert G. Haas., The expression of the complement regulators CD46, CD55, and CD59 by human sperm does not protect them from antispermatid antibody- and complement-mediated immune injury, *Fertility And Sterility*, 1993, 59 (4).
11. Yen-Pei Christy Chang, Xin Liu, James Dae Ok Kim, Morna A. Ikeda, Marnie R. Layton, Alan B. Weder, Richard S. Cooper, Sharon L. R. Kardia, D. C. Rao, Steve C. Hunt, Amy Luke, Eric Boerwinkle, and Aravinda Chakravarti ., Multiple Genes for Essential Hypertension Susceptibility on Chromosome 1q The American Journal of Human Genetics; 2007, Volume 80, 253- 264.
12. Cristina Menni, Sarah J. Metrustry, Georg Ehret, Anna F. Dominiczak, Phil Chowienczyk, Tim D. Spector, Sandosh Padmanabhan, and Ana M. Valdes., Molecular pathways associated with blood pressure and hexadecanedioate levels *PLoS One.*; 2017, 12(4): e0175479.
13. Qun Yan, Dongmei Sun, Xu Li, Guoliang Chen, Qinghua Zheng, Lun Li, Chenhong Gu, and Bo Feng., Association of blood glucose level and hypertension in Elderly Chinese Subjects: a community based study *BMC Endocr Disord.*; 2016, 16: 40.
14. Mukopadhyay etal., Effect of cryopreservation on sperm chromatin integrity and fertilizing potential in bovine semen, 2011, Volume 136, Issues 2-3, Pages 114– 121.
15. Nurcan Cetinkaya , Demet Ercin , Sümer Özvatan , Yakup Erel., Quantification of applied dose in irradiated citrus fruits by DNA Comet Assay together with image analysis. *Food Chemistry*; 2016, 192; 370–37
16. John R. Hofstetter, Aiwu Zhang, Aimee R. Mayeda, Tim Guscar, John I. Nurnberger, and Debomoy K. Lahiri., Genomic DNA from Mice: A Comparison of Recovery Methods and Tissue Sources. *Biochemical and Molecular Medicine*; 1997, 62, 197–202.