INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

*Research Article*

# COMPARATIVE STUDIES ON TUNGSTEN NANOPARTICLES SYNTHESISED BY CHEMICAL AND GREEN SYNTHESIS ROUTE FOR THEIR TOXICITY ASSAYS

**Ashish Kumar Sharma[1] and Manas Mathur[2*]**

[1]Department of Microbiology, Mewar University, Chittorgarh, Rajasthan-312 901, Jaipur, India.
[2]School of Agriculture, Suresh GyanVihar University, MahalRoad, Jagatpura, Rajasthan-302 017, Jaipur, India.

**ABSTRACT**

The present investigation has been performed to study effects comparative analysis of both biologically reduced and chemically synthesized nanoparticles on toxicities assays which shows its effects on male reproductive system. Albino Wistar male rats (*Rattusnorvegicus*), healthy fertile 3–4 months old weighing about 150–180 g were used for experimentation. NPs synthesis by both chemical and green route method and injected intra-peritoneal in healthy fertile male rats at the dose level of (4mg and 8mg/kg ) for 60 days. The characterization data has been validated by UV, SEM, TEM and XRD (data not shown). Animals were killed 24 h after withdrawal of treatment, and sperm, haematological, serum, tissue biochemistry, and histopathological analyses were performed. NPs were synthesised and their size confirmed by images like SEM, and TEM. Sperm analysis altered, hormonal, biochemical, and histological changes support its anti-spermatogenic effect on reproductive organ. Decrease in sperm density and motility, fertility index, testes and accessory organ weight, tissue fructose, and glycogen contents in testes were observed. Increased cholesterol level and decrease testosterone level showed its adverse effect on fertility. Histological degradation in testes is clearly visible. No significant alteration in body and vital organs weight was found. NPs were synthesised successfully and revealed contraceptive activity in male rats.

**Keywords**: Nanoparticles, Green synthesis, Anti-fertility, Sperm motility and Biochemical.

## INTRODUCTION

Nanotechnology is one of the very frontiers of science today. As a matter of fact, nanotechnology could affect us all, beyond nanoparticles, critical length scales and Nano tools: so, people should be able to see how all this science and technology could effect their lives ahead of the actual developments.

The applications of nanotechnology include various fields like optics, electronics, catalysis, bio-medicine, magnetics, mechanics, energy science, etc. Nano biotechnology is a multi-disciplinary field involving R&D of technology in different fields of science like biotechnology, nanotechnology, physics, chemistry, and material science [1, 2].Nanoparticles can be effectively used to deliver/transport relevant drugs to the brain overcoming the presence of blood–brain barrier [3, 4]. Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to enhance drug efficacy and reduces drug toxicity[5, 6]

Tungsten in particular, is considered as a potential candidate which exhilarates photo physical and photochemical properties[7,8,9,10]. Over the years, stoichiometric $WO_3$ of high quality has emerged as a potential candidate in electro chromic[11], photo chromic [12],thermo chromic, gas chromic and lithium battery [13]applications. Typically, $WO_3$ is a

technologically important wide band gap (~2·8 eV) oxide semiconductor system and recently it was shown that energy gap feature can be adequately varied with nitrogen doping content [14].Eco-friendly manner and green synthesis with use of biological agents like microorganisms and various plants could be replacement of chemical and physical methods Furthermore, the usage of plant biomass and extracts for synthesis of nanoparticles is possibly advantageous over microorganisms due to several factors such as simple handling procedures, readily scalability and preclusion of cell culture maintenance. However, the selection of plant material is very critical in obtaining the best reducing agents in order to produce excellent size and shape of nanoparticles [15].biogenic synthesis is not only reduced environmental impact, but also can produce large amount of nanoparticles that do not have contamination and have perfect size and morphology. Biosynthetic routes can actually provide nanoparticles of a better defined size and morphology than some of the physicochemical methods of production [16]. Plant contains a complex network of metabolites and enzyme that can be manipulated to synthesize nanoparticles. The basic chemical components of these plants are flavonoids, flavonoids glycosides and phenylpropanoid glycosides. The presence of various chemical compounds in plant such as polyphenols, flavonoids, sterols, and triterpenes, reducing sugar like glucose and fructose, and protein could help to produce metallic nanoparticles.

The explosion of population is a global problem that poses significant threat to the quality of life in all our World. To combat this grave situation, effective family planning is required with equal participation of both males and females. Though some modalities have been developed as a contraceptive agent for the females, yet no effective and reversible systemic method has been developed to date for the males. Induction of acuteoligospermia or azoospermia is possible by using hormonal steroid or gonadotropic-releasing hormone (GnRh) agonist/antagonist. These methods are either irreversible or affect libido and secondary sex characteristics [17]and supplementary treatment is required rendering these methods cost-ineffective. Another way would be to find effective anti-spermatogenic agents that could rely on alternative means to the hypothalamo-pituitarygonadal axis, more particularly, compounds primarily designed for the purpose of other clinical use unrelated to contraception but unexpectedly revealed to have antifertility effect upon routine evaluation. With the use of present-day knowledge of synthesize. Np to synthesize different analogues and after careful pharmaco-toxicological surveillance, it is possible to achieve some useful products having no overt toxicity.

**MATERIALS AND METHODS**

Healthy wistar albino male rats (*Rattusnorvegicus*) weighing about 150-180 g were taken for experiments. They were housed in clean and hygienic polypropylene (8"x12"x8") cages and were maintained under standard conditions. The animals were maintained with standard pellet feed (Ashirwad food Chandigarh India) and water provided ad libitum. Animal ethics committee (IAEC) and conducted according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India.The duration of experiment was set for 60 days. Control animals received a similar volume of vehicle (0.5ml distilled water per day) for 60 days.

The experiment comprised 5 groups of six animals in each group. In group I, rats received vehicle only, in group II rats were given 4 $mgkg^{-1}$body weight dose of nanoparticles, in group III rats were given 8 $mgkg^{-1}$ body weightdose plant (both biology reduced nanoparticles). In group IV rats were give 4 $mgkg^{-1}$ body weight dose of nano particle, in group V rats were given 8 $mgkg^{-1}$ body weight dose of nano particle (chemically synthesized nano particle)At end of experiment period, animals of control, treatment groups were weighed and killed under ether anaesthesia after 24 h of the termination of respective treatments.

Body weight was recorded at the beginning and last day of treatments. Organ weights were recorded after autopsy and represented as weight per 100 g body weight. Blood was collected from cardiac puncture and serum was separated at 3000 rpm by centrifugation and stored at -20°C until used for the biochemical assays. The testes, epididymis, seminal vesicle and ventral prostrate were dissected out and freed from adherent tissues and blood. Caudaepididymal sperm density and motility were observed according to the procedure [18]. Tissue (100g) was taken in 1 ml of normal saline for sperm motility and density and some drops of mixed sample were taken on the neubauer counting chamber. The percentage motility was determined by counting both motile and immotile spermatozoa per unit area.

Testes were carefully dissected out following abdominal incision and fixed in 10% formalin and processed routinely for paraffin embedding. Sections were obtained with a rotary microtome , stained with haematoxylin and eosin stain

(H/E) and observed under a light microscope.The values are expressed as mean ±SEM of six observations in each group. All group were subjected to one way analysis ofvariance (ANOVA), which was followed by bonferroni post-hoc test. Results were considered and compared at the three levels of significance i.e. p≤0.05, p≤0.01 and p≤0.001 levels.

**RESULTS AND DISCUSSION**

In the present investigation we observed comparison of body weights between untreated (vehicle control) and suspensions treated groups. The results of this study revealed that there were no significant differences found between the initial and final body weight treated with group II,III, IV,V when compared with vehicle control (group I). (**Table 1)**

On the other hand , significant (p≤0.05,p≤0.01,p≤0.001) reductions were noted in the weights of the testis, epididymis, seminal vesicle and ventral prostrate in nanoparticles treated rats when compared with vehicle control (**Table** 1). Sperm motility declined by 13.00, 39.08, 24.53 and 52.15 in groups II, III, IV, and V, respectively in comparison to control groups **(Table 2).** Sperm density in testes and cauda epididymis was also reduced significantly in group II (p≤0.05), group III (p≤0.05, p≤0.001), group IV (p≤0.05, p≤0.001) and group V(p≤0.001) compared to control animals (table).Concentrations of testosterone , LH and FSH in serum decreased significantly in treated groups (p≤0.001, p≤0.01,p≤0.05) in comparison with the control group (**Table2**).

In the animals of groups II, III and V the cholesterol level in testes showed highly significant increase at p≤0.001 level of significance when compared to control group whereas group IV showed significant increase at p≤0.05 level of significance when compared to control group (**Table 3).**The glycogen content of testes was significant in groups II, and IV (p≤0.01) and highly significant in group III and V (p≤0.001) in comparison to control group. (**Table 3**).The seminal vesicular fructose significantly decreased in groups II, III and IV (p≤0.01) whereas in group V the level of fructose decreased highly significantly (p≤0.001) in comparison to control group (**Table 3)**

The histoarchitacture of testes of control rats showed normal round or oval seminiferous tubules with active spermatogenesis. Histopathology examination of testes of groups III and V revealed degeneration in spermatogonial cell lining the seminiferous tubules associated with incomplete spermatogenesis and sloughing of degenerated germ cells histopathology of testes of group II,IV animals showed less damaged seminiferous tubules as compared to the control group **(Fig. 1-5).**

Androgens play a crucial role in the development of male reproductive organs such as epididymis, vas deferens, seminal vesicle and testes. Androgens specially testosterone with high levels are necessary for active spermatogenesis [19]. The efficiency of spermatogenesis and fertility are increased in males as a result of synergistic effect of FSH and testosterone whereas LH stimulates spermatogenesis through stimulating testosterone production in lyedig cells. It has been considered that low levels of FSH and LH prevent gonads from either producing sperms or insufficient amount of testosterone production[20].

The other important factors other than androgens that effect spermatogenesis are sperm motility and density. Lowering of motility and density suggested an undersupply of testosterone to the epididymis which directly affects the activity of spermatogenesis [21].Fructose concentration also has been considered to be essential for motility and its viability as an energy source.Administration of the compound decreased the concentration of testosterone, FSH and LH in serum which might be due to the disturbances in the spermatogenic activity. The significant reduction in weights of accessory glands indicates the decrease in testosterone level and inhibition of spermatogenesis [22].

The histopathological changes in testes such as incomplete spermatogenesis and sloughing of degenerated germ cells support deleterious effects on male fertility of the treated groups. Sloughing of germ cells of treated groups compared to control group indicates testicular dysfunction.Alterations in male fertility and decrease in fertility parameters resulted in abnormal sperm functions which gave rise to sterility.

**CONCLUSION**

The decreased levels of sperm density, motility weight of reproductive organs and clumping of spermatozoa in the seminiferous tubules reveals the antifertility activity of mice treated with both biologically reduced and chemically synthesised nanoparticles. The nanoparticles synthesised by green route method also affected the serological parameters like serum cholesterol, protein. There was significant effect on cholesterol, protein content of testes and also fructose level in seminal vesicle have significant

effect in treated rat.The antifertility activity of group III was higher than to the group II and group V was higher than to the group IV when the compare with control rats.Our research showed that oral administration of nanoparticles synthesised by green route lead to a dose as well as time duration dependent defects in the testicular spermatogenesis which lead to production of defective sperms.The nanoparticles prepared by green route method could thus be recommended, after extensive laboratory and clinical trials as reversible herbal male contraceptive , especially that it showed a high safety margin.This study may be helpful for future investigation in the formulation of male contraceptive and specific chemical entity elucidation responsible for antifertility.

**ACKNOWLEDGMENTS**

The authors are highly thankful to Mewar University for providing necessary facilities and Head, Department of Zoology, University of Rajasthan for providing permission to carry pot research on albino mice.

**Table 1:**

| Groups | Body Weight (g) | | Organs Weight (mg/100g body weight) | | | |
|---|---|---|---|---|---|---|
| | Initial | Final | Testes | Epididymides | Seminal Vesicle | Ventral Prostate |
| Group I | 219.00±5.325 | 251.00±14.95 | 926.07±10.12 | 314.74±6.34 | 679.61±21.00 | 322.04±9.97 |
| Group II | 227.00±8.00 | 260.00±11.17 | 701.12±40.10$^{a}$ | 278.83±9.67 $^{a}$ | 588.82±28.17$^{a}$ | 278.89±12.40 $^{a}$ |
| Group III | 230.00±9.25 | 272.00±4.89 | 412.02±56.16$^{c}$ | 267.45±10.41$^{b}$ | 553.48±18.75$^{b}$ | 250.22±9.46$^{b}$ |
| Group IV | 251.00±4.67 | 280.00±7.39 | 677.42±39.42$^{a}$ | 260.11±11.32$^{b}$ | 590.12±27.10$^{a}$ | 276.35±11.55$^{a}$ |
| Group V | 240.00±5.89 | 275.00±6.46 | 280.17±32.53$^{c}$ | 227.57±6.53$^{c}$ | 560.17±21.61$^{b}$ | 253.40±8.17$^{b}$ |

Values are expressed as mean ± SEM (n= 6); ns= non-significant. Levels of significance: $^{a}$ p<0.05; $^{b}$ p<0.01; $^{c}$ p<0.001 compared with group I.

Group I: Control group; Group II: 4mg/kg body weight; Group III: 8mg/kg body weight (biology reduced nanoparticles )

Group IV: 4mg/kg body weight, Group V: 8mg/kg body weight ( chemically synthesized Nano particle)

**Table 2:**

| Groups | Sperm Motility (%) | Sperm Density (millions ml$^{-1}$) | | Testosterone | LH | FSH |
|---|---|---|---|---|---|---|
| Caudaepididymides | Testes | Caudaepididymides | (ng/dl) | | | |
| Group I | 76.45±1.93 | 5.42±0.31 | 41.32±1.69 | 3.447±0.402 | 3.42±0.373 | 0.253±0.0321 |
| Group II | 66.51±1.24$^{a}$ | 4.29±0.32$^{a}$ | 35.47±1.46 $^{a}$ | 2.047±0.249 $^{a}$ | 2.303±0.421$^{ns}$ | 0.122±0.0189 $^{a}$ |
| Group III | 46.67±2.97 $^{b}$ | 4.18±0.46$^{a}$ | 22.17±1.07$^{c}$ | 1.522±0.322 $^{b}$ | 2.212±0.159 $^{a}$ | 0.118±0.0140 $^{b}$ |
| Group IV | 57.69±2.33$^{b}$ | 2.78±0.190$^{c}$ | 36.52±1.26$^{a}$ | 2.068±0.261$^{a}$ | 2.019±0.105$^{a}$ | 0.127±0.0176$^{a}$ |
| Group V | 36.58±1.92$^{c}$ | 2.69±0.342$^{c}$ | 20.10±1.06$^{c}$ | 1.079±0.078$^{c}$ | 1.890±0.276$^{b}$ | 0.116±0.0126$^{b}$ |

Values are expressed as mean ± SEM (n= 6); ns= non-significant. Levels of significance: $^{a}$ p<0.05; $^{b}$ p<0.01; $^{c}$ p<0.001 compared with group I.

Group I: Control group; Group II: 4mg/kg body weight; Group III: 8mg/kg body weight,

Group IV: 4mg/kg body weight, Group V: 8mg/kg body weight (Nano particle)

**Table 3: Tissue biochemistry**

| Groups | Cholesterol (mg/g) | Glycogen (mg/g) | Fructose (mg/g) |
|---|---|---|---|
| | Testes | Testes | Seminal Vesicle |
| Group I | 7.76±0.12 | 4.96± 0.21 | 3.83± 2.96 |
| Group II | 8.88± 0.22$^{c}$ | 3.89± 0.23$^{b}$ | 2.96± 0.01$^{b}$ |
| Group III | 10.07±0.29 $^{c}$ | 3.13± 0.25$^{c}$ | 2.87± 0.19$^{b}$ |
| Group IV | 7.82±0.09$^{a}$ | 3.90±0.19$^{b}$ | 2.59±0.17$^{b}$ |
| Group V | 12.02±0.18$^{c}$ | 2.87±0.15$^{c}$ | 1.97±0.18$^{c}$ |

Values are expressed as mean ± SEM (n= 6); ns= non-significant.

Levels of significance: $^{a}$ p<0.05; $^{b}$ p<0.01; $^{c}$ p<0.001 compared with group I.

Group I: Control group; Group II: 4mg/kg body weight; Group III: 8mg/kg body weight,

Group IV: 4mg/kg body weight, Group V: 8mg/kg body weight(Nano particle)

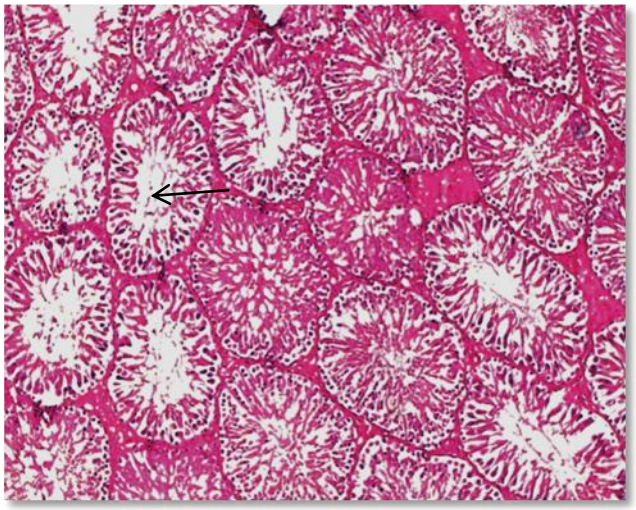

**Fig. 1: Photomicrograph rat testis Control showing interstial tissues (IT) and seminiferous tubules (ST) with normal germ cells and spermatogenesis**

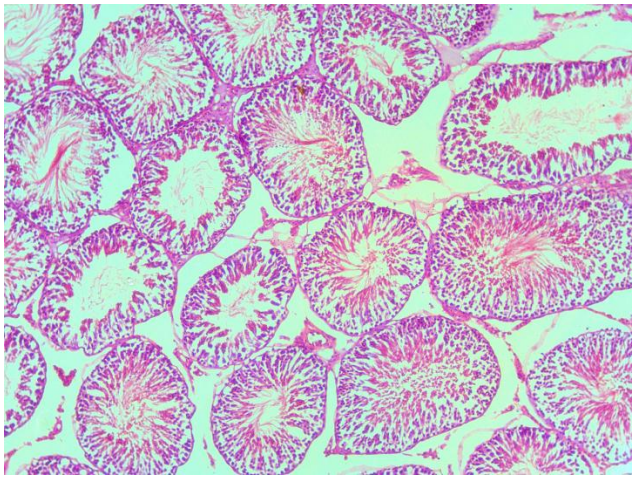

**Fig. 2: (4mg/kg dose of biologically reduced nanoparticles b.wt.) Photomicrograph rat testis showing degenerative changes in interstitial tissues (IT)**

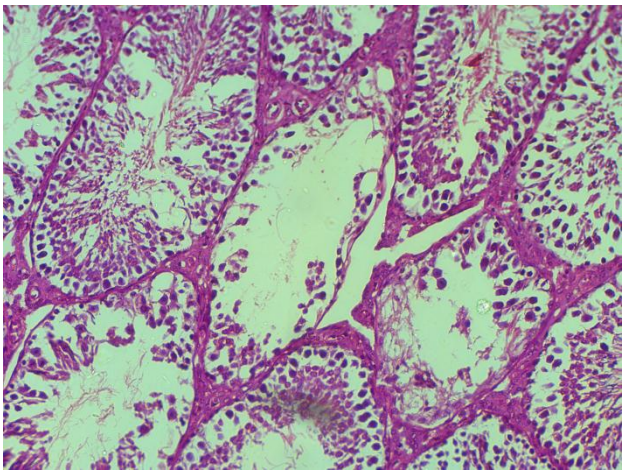

**Fig. 3: (8mg/kg dose of biologically reduced nanoparticles b.wt.) Photomicrograph rat testis showing Disappearance of germ cells and sertoli cells (SC), spermatogenesis irregular**

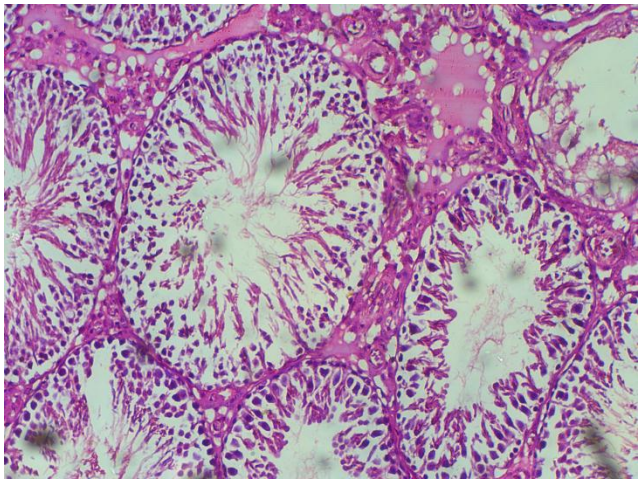

**Fig. 4: (4mg/kg dose of chemically synthesized nanoparticlesb.wt. nanoparticle) Photomicrograph rat testis showing germ cells in seminiferous tubules(ST) decreased sharply, vacuolization of germ cells**

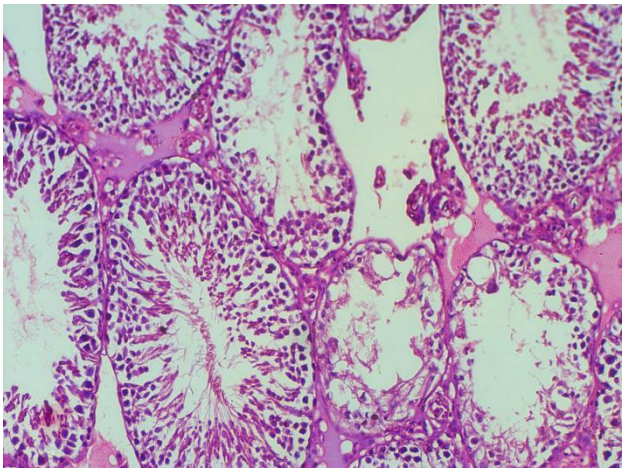

**Fig. 5: (8mg/kg dose of chemically synthesized nanoparticles b.wt.nanoparticle) Photomicrograph rat testis showing highly degeneration of seminiferous tubules (ST) and decreased amount of mature spermatozoa in lumen(L).**

**REFERENCES**


1. Rai M, Yadav A and Gade A. Current trends in phytosynthesis of metal nanoparticles. Cri Rev. Biotechnol. 2008;28:277–284.
2. Huang J, Chen C, He N, Hong J, Lu Y, Qingbiao L, Shao W, Sun D, Wang XH, Wang Y and Yiang X. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomumcamphora leaf. Nanotechnol. 2007;18:105–106.
3. Alyautdin RN, Tezikov EB, Ramge P, Kharkevich DA, Begley DJ and Kreuter J. Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study. J Microencapsul. 1998;15 : 67–74.
4. Garcia-Garcia E, Gil S, Andrieux K, Desmaële D, Nicolas V, Taran F, Georgin D, Andreux JP, Roux F and Couvreur P. A relevant in vitro rat model for theevaluation of blood–brain barrier translocation of nanoparticles. Cell Mol Life Sci. 2005;62:1400–1408.
5. Feng SS, Mu L and Win KY. Nanoparticles of biodegradable polymers forclinical administration of paclitaxel. Curr Med Chem. 2004;11:413–424.
6. De Kozak Y, Andrieux K, Villarroya H, Klein C, Thillaye-Goldenberg B and Naud MC. Intraocular injection of tamoxifen-loaded nanoparticles: a new treatment of experimental autoimmune uveoretinitis. Eur J Immunol. 2004;34:3702–3712.
7. Lel J K and Bard A J. Electrochemical investigation of the electron-transferkinetics and energetics of illuminated tungsten oxide colloids. J Phy Chem.1987;91:5083-5087.
8. Henglein A. Small-particle research: physicochemical properties of extremely small colloidal metal and semiconductor particles. Chem Rev. 1989;89:1861-1873.
9. Hagfeldt A and Gratzel M. Light-Induced Redox Reactions in Nano crystalline Systems. Chem Rev.1995;95:49-68.
10. Li ST, Germanenko IN and Shall MSEI. Semiconductor Nanoparticles in Contact: Quenching of the Photoluminescence from Silicon Nanocrystals by $WO_3$ Nanoparticles Suspended in Solution. J Phy Chem B. 1998; 102:7319-7322.
11. Granqvist CG. Electrochromic tungsten oxide films: Review of progress (1993-1998). Solar Ener. Mat Solar Cells. 2000;60:201-262.
12. Sun M, Xu N, Cao YW and Yao JN and Wang EG. Nanocrystalline tungsten oxide thin film: preparation, microstructure, and photochromic behaviour. J Mat Res. 2000;15:927-933.
13. Lee K, Seo WS and Park JT. Synthesis and Optical Properties of Colloidal Tungsten Oxide Nanorods.J. Amer Chem Soc. 2003;125: 3408-3409.

14. Lethy KJ, Pandya S, Beena D, Vinodkumar R, Sathe V and Pillai VPM . J Phy App Phys. 2009;42: 185407.
15. Saxena M, Saxena J and Pradhan A. Flavonoids and phenolic acids as antioxidants in plants and human health. Int J Pharma Sci. Res. 2012;16:130–134.
16. Ravindran A, Chandran P and Khan SS. Bio functionalized silver nanoparticles: Advances and prospects. Collo. Surf. Bio inter. 2013; 105:342–352.
17. Paulsen CA, Christensen RB and Bagatell CJ. Status of male contraception:hormonal approach. In: Corson SL, Derman RJ, Tyner LB, editors. Fertility control. 2nd ed. London: Golden Publishers; 1994;281–92.
18. Prasad MRN, Chinoy NJ and Kadam KM. Changes in succinate dehydrogenase levels in the rats epididymis under normal and altered physiological condition. Fert Steril. 1972;23:186–190.
19. Dohle GR , GM Colpi , TB Hargreave , GK Papp , Jungwirth A and Weidner W. EAU Guidelines on Male Infertility. European Urology. 2005; 48:703–711.
20. Nieschlag E and Nieschlag S. Testosterone deficiency: a historical perspective. Asian J Androl. 2014; 16: 161–168.
21. William H Walker. Non-classical actions of testosterone and spermatogenesis. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2010; 365:1546.
22. Rehm S, White TE, Zahalka EA, Stanislaus DJ, Boyce RW and Wier PJ. 2008. Effects of Food Restriction on Testis and Accessory Sex Glands in Maturing Rats. Toxico Pathol. 2008; 36:687-694.