The Journal of Phytopharmacology 2024; 13(4):341-344
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2024; 13(4): 341-344
July- August
Received: 28-05-2024
Accepted: 22-08-2024

doi: 10.31254/phyto.2024.13411

Mistu Tripura
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Bhupesh Kamdi
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Ranjit Ingole
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Maheshkumar Ingawale
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Prajakta Kuralkar
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Sunil Hajare
Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola-444104, Maharashtra, India

Correspondence:
Dr. Sunil Hajare
Department of Veterinary Pharmacology & Toxicology, Post Graduate Institute of Veterinary & Animal Sciences, Krishinagar, Akola- 444104, Maharashtra, India
Email: sunilhajare@mafsu.in

Effect of Lemon peel extract mediated gold nanoparticles on hematobiochemical and sperm parameter alterations in lead- arsenic induced combined exposure in male rats

Mistu Tripura, Bhupesh Kamdi, Ranjit Ingole, Maheshkumar Ingawale, Prajakta Kuralkar, Sunil Hajare

ABSTRACT

Gold was used in the vedic era in India to enhance strength, potency and to combat the aging process in humans. It is one of the few metals which can be used at nano scale due to its resistance to oxidation. Considering the biological activity of gold, the present study was carried out to evaluate the effect of Lemon peel extract gold Nanoparticals (LPGNP) on As + Pb induced reproductive toxicity in rats. In present study twenty-four wistar rats were divided into four groups, group I served as control, group II, III, IV received co-exposure of Sodium Arsenate 13.8mg/kg and Lead acetate 116.4 mg/kg, p.o. daily for 14 days, followed by oral administration of LPGNP @10 and 20 mg/kg, to group III and IV, respectively for 6 weeks. At the end of experiment hematology, reproductive parameters and histopathology of testes was studied. The findings of study revealed significant alteration in hematobiochemical parameters, serum testosterone concentration, sperm motility, total sperm count and sperm viability in rats received As-Pb exposure whereas LPGNP administration caused marked improvement in reproductive parameters. In histopathology of testis, As + Pb caused degenerative changes of seminiferous tubules, and sloughing of spermatogenic cells. In LPGNP treated rats, minimal histopathological alterations were observed in testis. In conclusion LPGNP caused significant improvement in biochemical and sperm parameters and testosterone level in As + Pb induced reproductive toxicity in rats.

Keywords: Nanoparticles, LPGNP, Lemon peel, Arsenic, Lead, Sperm.

INTRODUCTION

Gold is thought to be the best rejuvenator and its medical indications can be found in all ancient medical classics. In Ayurveda Swarnabhasma (gold ash) is found to have been developed since the time of Nagarjuna (800AD) and mainly used as an aphrodisiac, to enhance vigour, vitality, memory, immunity, and longevity. Lemon (*Citrus lemona*) fruit is a rich source of flavonoid glycosides, coumarins. The peel extract of lemon acts as reducing and capping agent for the preparation of gold nano particles and therefore bioactive and stable gold nanoparticles can be prepared from gold salt.

Arsenic and lead are the multi-organ toxicants and endocrine disrupting nonessential metal present ubiquitously in environment and biota as pollutant. There are several reports depicting their adverse effect on reproductive functions. Previous studies demonstrated that both these heavy metals reduces serum testosterone level, sperm number, sperm viability and thus affecting the male reproduction [1,2]. Arsenic a major toxic metalloid found to be existed in air, water and soil. Its exposure to higher-than-acceptable level occurs either in workplace, e.g., in smelting industries, coal-fired power plants, cosmetic industries, agriculture, etc, or through arsenic-contaminated food or drinking water, particularly the contaminated groundwater has caused severe health hazards in the exposed population[3]. Lead plays a significant role in modern industry where a wide variety of population bears a risk of occupational exposure beside it is a harmful environmental pollutant commonly absorbed from respiratory system, digestive system and can also be absorbed from the skin. Lead is recognized as multi-organ toxicant that affect liver, kidney, reproductive system and other physiological organs [4] . As arsenic and lead are common heavy metal environmental toxicants, their co-exposure could be highly hazardous which requires considerable attention and detail toxicity study.

Considering remarkable rejuvenating and reproduction enhancing properties of gold, the present research study was undertaken to evaluate the effect of *lemon peels* extract mediated gold nano particles on lead and arsenic co-exposure induced subacute toxicity in wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals

The reagents and chemicals used in the study were of analytical grade. $NaAsO_2$ (Sodium Arsenite) and $HAuCl_4$ (Gold salt) were obtained from Hi-Media India, lead acetate was obtained from SD fine Chemicals, India.

Animals

The experiment was conducted on twenty-four healthy adult Wistar rats of 160 to 190g. Animals were acclimatized for a week before experiment. The experimental animals were housed in polypropylene cages and provided with rat pellet chow (Nutrivet life Sciences, Pune), pure drinking water ad-libitum and 12-12 h cycle of darkness and light. The study protocol was approved (approval no. 312/01/2000/02/22 dated 08.04.2022) from IAEC (Reg. No. 312/GO/ReBi/2000/CPCSEA) of PGIVAS, Akola.

Plant Extraction and Biosynthesis of gold nanoparticles

The dried lemon peel powder is used for the preparation of 20% lemon peel extract as per the procedure described earlier[5]. The 20% lemon peel extract solution is added to 2 mM of HAuCl4 solution in the ratio of 2:1 with constant stirring. The gold nanoparticles fabrication happens quickly within 10 minutes. The color change of the solution of HAuCl4 from yellow to ruby red indicates the formation of gold nano particles. In this process lemon peel extract acts as a reducing and capping agent for the synthesis of stable gold nanoparticles in the range of 1-100 nM in size with average size of 20nM [5].

Animal Treatment

In vivo study conducted in 24 male wistar rats divided equally into four groups (Table 1). The animals were procured from the Laboratory Animal Resource Section of PGIVAS, Akola. The ambient temperature of 25±2 °C and 12- hour Light-dark cycle was maintained during the experiment. The relative humidity was 45-60 % during the experiment. The animals fed with pelleted feed procured from Nutrivet life Sciences, Pune and water provided *ad libitum.* The animals were housed in polypropylene cages 5 days prior to experimentation as a period of acclimatization. The subacute toxicity was induced with Sodium Arsenate and Lead acetate administered @13.8mg/kg and 116.4 mg/kg, p.o. respectively to wistar rats for 14 days followed by LPGNP supplementation @10 and 20 mg/kg, p.o. for 6 weeks.

Table 1: Different groups of animal, treatment, dose and duration of experiment

Groups	Treatment	No. of animals	Duration of experiment
I	Control – NS/DW p.o for 8 weeks	6	8 weeks
II	Sodium Arsenite 13.8mg/kg body weight + Lead acetate @116 mg/kg body weight for 14 days	6	8 weeks
III	Sodium Arsenite 13.8mg/kg body weight for 14 days + Lead Acetate 116 mg/kg body weight for 14 days + LPGNP 10mg/kg from 15th day till last day of experiment	6	8 weeks
IV	Sodium Arsenite 13.8mg/kg body weight for 14 days + Lead Acetate 116 mg/kg body weight for 14 days + Lemon peel extract LPGNP 20mg/kg from 15th day till last day of experiment	6	8 weeks

Haematobiochemical analysis

The effect LPGNPs was evaluated on haematobiochemical parameters (Hb, TEC, PCV, MCH, MCHC and TLC) of all animals of different groups.

Sperm parameters

Immediately after sacrifice cauda epididymis from each male animal was collected and minced in prewarmed 10 ml of 0.1 M phosphate-buffered saline (pH 7.2). Counting of sperms was done with 100x magnification using a compound light microscope. Counting (on both sides of the hemocytometer) was performed on all 25 large squares at each counting chamber and different sperm parameters viz. sperm motility, sperm viability, total and abnormal spermatozoa count were estimated as per the standard methodology[6,7] .

Testosterone assay

The testosterone estimation was carried out at Department of Nuclear Medicine, Mumbai Veterinary College Mumbai (MS, India). The hormone level in male rats was estimated following "COAT-A-COUNT" method of Radio Immune Assay (RIA) by using kit procured from "Immunotech SAS" Beckman Coulter Co., Mereille Cedex-9, France.

Histopathological examination

Animal were sacrificed at the end of experiment and testes of rats from different groups were collected in neutral buffered formalin (10%). After fixation, tissues were processed for histopathology using alcohol and xylene followed by impregnation in paraffin wax (Qualigen) as per routine method. Impregnated tissues were sectioned in 5µ slices and stained with H & E stain as per the method described by Luna, (1968). Further well stained slides were observed under the microscope for histopathological alterations.

Statistical analysis

Data was analyzed using one way ANOVA followed by Tukey's post hoc test in IBM SPSS software, Version 22. Significance was observed at 5% level.

RESULTS

The animals in Group II treated with Arsenic and lead showed mild aggressiveness, stressed and mild anorexia during the initial weeks i.e. upto 3rd week of experiment. The body weight gain in the toxic control groups was seen significantly ($p<0.05$) decreased from 1st week upto 3rd week. From fourth week onwards no significant decrease in the body weight was observed in As-Pb toxic group. The body weight gain in all LPGNP treated experimental animals was in the normal range as compared to control. There were no significant alterations in different heamatological parameter observed between control and different treatment groups except TEC and TLC values (Table 2). In As+Pb exposed toxic Group-II the TEC and TLC values decreased significantly ($p<0.05$) whereas significant and dose dependent improvement in TEC and TLC was observed from LPGNP treatment groups III and IV.

Table 2: Heamatological values (Hb, TEC, PCV, MCH, MCHC and TLC) related to erythrocytes in control and different treatment group of rats

Groups	Hb (g/Dl)	TEC (10^6cumm)	PCV (%)	MCH (pg)	TLC 10^3/cumm
I	13.80±1.21	6.97±0.29^{a}	29.42±2.48	17.82±0.70	8.21±0.51^{d}
II	12.14±1.12	3.94±0.4^{cd}	31.16±1.10	17.24±0.69	14.96±0.97^{a}
III	12.45±0.99	4.63±0.37^{bc}	31.13±1.42	16.01±0.74	11.39±0.76^{b}
IV	13.43± 0.96	5.71±0.33^{b}	30.61±1.16	17.70±0.53	10.12±0.73^{bc}
*Sign	NS	*	NS	NS	*

Values indicate mean ± S.E., n=6, Significance level * P<0.05, NS- Non-Significant

Table 3: Serum AST, ALT, ALP, Creatinine and BUN Values in control and different treatment group of rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dl)	BUN (mg/dl)
I	89.59±3.59^{b}	35.23±4.29^{b}	38.77±2.91^{b}	1.12±0.05^{c}	17.10±1.03^{c}
II	160.14±13.52^{a}	52.71±2.26^{a}	136.63±12.93^{a}	1.72± 0.17^{ab}	32.32±1.63^{a}
III	111.16±16.24^{b}	35.05±3.67^{b}	53.29±2.10^{b}	1.52±0.18^{bc}	24.62±1.17^{b}
IV	91.19±8.90^{b}	27.67±2.50^{c}	52.66 ±1.68^{b}	1.56± 0.15^{bc}	24.16±1.05^{b}
*Sign	*	*	*	*	*

Values indicate mean ± S.E. n=6, Significance level * P<0.05

Table 4: Effect of lemon peels extracts gold nano particles in subacute toxicity of lead, arsenic and their combination on male reproductive sperm parameter in wistar rats

Groups	Sperm Motility %	Total Sperm count spermatozoa/ml	Total Sperm Abnormality %	Sperm viability %	Hormone Testosterone (ng/dL
I	61.50±1.85^{ab}	219.13 ± 8.77^{a}	4.83 ± 0.47^{d}	83.67 ±1.72^{a}	3.13 ± 0.24^{a}
II	51.66±2.04^{cd}	123.13 ± 7.69^{d}	17.16 ±1.30^{a}	52.00± 2.08^{c}	1.08 ± 0.12^{c}
III	58.16 ±2.83^{abc}	203.06±15.95^{ab}	5.50± 0.76^{bc}	63.66± 1.87^{b}	2.59± 0.21^{b}
IV	64.00 ±1.67^{a}	183.86±20.31^{bc}	6.83±0.90^{b}	65.00±2.03^{b}	2.96 ±0.21^{ab}
*Sign	*	*	*	*	*

Values indicate mean ± S.E. n=6, Significance level * P<0.05

As-Pb intoxicated animals showed significant (p<0.05) increase in AST, ALT and ALP whereas the treatment with LPGNPs shows retrieval effect on alteration of AST, ALT and ALP (Table 3). The rats exposed with arsenic + lead revealed mild numerical increase in mean serum creatinine and suggest milder nephrotoxicity. Treatment with LPGNPs showed retrieval in the creatinine values towards normal in groups III and IV. The serum BUN values of control and different groups differ significantly. There is marked increase in serum BUN of group II rats while significant improvement was observed after LPGNPs treatment.

The sperm parameters viz sperm motility, total sperm count, and sperm viability decreased significantly (p<0.05) in As-Pb intoxicated animals of group II whereas total sperm abnormalities found to be increased as compared to control group (Table 4). The treatment with LPGNPs caused significant recovery in sperm parameters in both treatment groups. Lead-arsenic intoxication caused marked depletion in serum testosterone concentration in group II rats. The testosterone concentration found to be retrieved significantly (p<0.05) in gold nano particle treated groups.

In histopathological examination tissue sections of testis from control group rats showed normal architecture (Fig 1) and group II tissue sections showed increase intertubuler spaces and reduction in the size of tubule, degenerative changes of seminiferous tubules, widening of interstitial spaces, derangement, sloughing of spermatogenic cells (Fig 2). In treatment groups receiving LPGNPs histological changes as aforesaid were observed minimal (Fig 3 & 4).

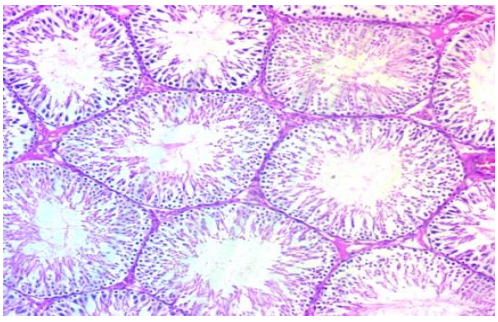

Figure 1: Section of testes (Group I) showing organised basement membrane, normal Leydig cells and spermatids/spermatozoa in lumen (H&E, 100X)

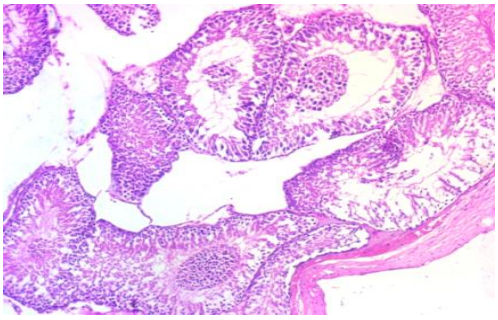

Figure 2: Section of testes (Group II) showing increase intertubuler spaces and degenerative changes in seminiferous tubules, and derangement, sloughing of spermatogenic cells in lumen (H&E, 100X)

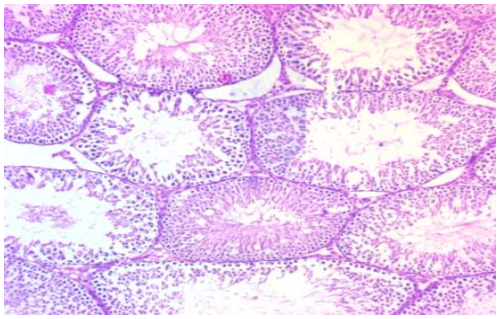

Figure 3: Section of testes (Group III) showing mild to moderate degeneration of germinal cells in few seminiferous tubules (H&E, 100X)

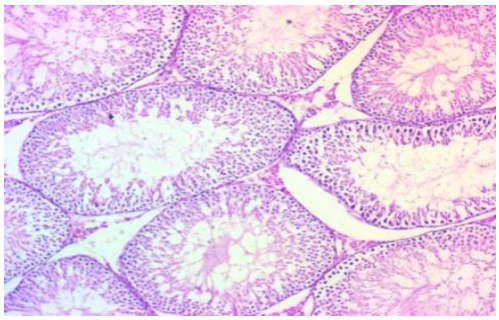

Figure 4: Section of testes (Group IV) showing mild degenerative changes in few seminiferous tubules (H&E, 100X)

DISCUSSION

Gold is used in Indian traditional medicine in various forms since ancient time for disease conditions like psychosomatics disorders, cancer, arthritis, loss of vigour and vitality[8]. The gold was used in the form of ash (bhasma) since 8th century AD after proper incineration as depicted in the Ayuveda. The gold is considered suitable be used at nano scale due to its resistance to oxidation. In the present study green synthesized LPGNP at 10 and 20 mg/kg administered orally to rats for six weeks did not show any adverse effect and there is no change in food and water intake. It is considered to be one of the safe metals used in therapeutics though not much work has been done to explore its toxicity potential. LPGNP at 10 and 20 mg/kg is non-toxic supported by earlier report in which gold nano particles showed no evidence of toxicity in acute, sub-acute and chronic toxicity study conducted in mice [9].

Arsenic + lead combined toxicity showed adverse clinical effects at the doses employed in this study(As @13.8mg/kg and Pb@116.4 mg/kg, p.o.) which is supported by earlier reports. The increased liver function marker enzymes ALT, AST, and ALP in serum are basic indicator of hepatotoxicity. The significant rise in the levels of ALT, AST, and ALP in Arsenic-Lead administered rats suggesting cellular leakage of these enzymes. As+Pb also caused marked increase in BUN which may be due to decrease glomerular filtration suggesting mild to moderate nephrotoxicity. Similar to present study hepatorenal marker enzyme alterations are observes by earlier researchers in arsenic + lead co-exposure group[10]. As+Pb caused marked depletion serum testosterone but significant lesser alteration in serum testosterone was observed in treatment groups which may be due to antioxidant and rejuvenating action of LPGNPs. Several authors reported that As-Pb cause alterations in various sperm parameters and testosterone are consistent with the findings of present study[11,12]. The histological alterations induced by As-Pb in testis are align with earlier reports of individual toxicity study of As and Pb[13,14]. LPGNPs treated groups showed marked recovery indicating beneficial effects of LPGNPs on reproductive system in As-Pb induced toxicity. The LPGNP at 10 and 20 mg/kg employed in this study showed similar effects with no additional or significant benefit of using the dose of 20 mg/kg which suggest that LPGNP at 10 mg/kg is the beneficial dose for hematobiochemical and reproductive parameters as per the outcome of present study conducted in wistar rats.

CONCLUSION

From the results of the present investigation, it is concluded that, Pb and As co-exposure caused marked alterations in hematobiochemical and reproductive parameters. Upon LPGNPs oral administration significant restorative effect on sperm parameters, testosterone assay and histomorphology of testis was encouraging which validates the Ayurvedic and traditional use of gold as rejuvenator and reproduction enhancer in males which further suggests broader research on gold nanoparticle before its possible application in reproductive disorders.

Acknowledgments

The author acknowledges Post Graduate Institute of Veterinary & Animal Sciences, Akola (affiliated to University MAFSU, Nagpur), for funding the research.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial Support

None declared.

REFERENCES

1. Lima NG, De Sousa DP, Pimenta FCF, Alves MF, De Souza FS, Macedo RO, Cardoso RB, De Morais LCS, Margareth de Fátima F, De Almeida RN. Anxiolytic-like activity and GC–MS analysis of (R)-(+)-limonene fragrance, a natural compound found in foods and plants. Pharmacology Biochemistry and Behavior. 2013;103(3):450-454.
2. Sainath SB, Meena R, Supriya CH, Reddy KP, Reddy PS. Protective role of Centella asiatica on lead-induced oxidative stress and suppressed reproductive health in male rats. Environmental toxicology and pharmacology. 2011; 32(2): 146-154.
3. Chakraborti D, Singh SK, Rahman MM, Dutta RN, Mukherjee SC, Pati S and Kar PB. Groundwater arsenic contamination in the Ganga River Basin: a future health danger. Int. J. Environ Res. Public Health. 2018; 15(2): 180, doi: 10.3390/ijerph15020180.
4. Ezejiofor AN, and Orisakwe OE. The protective effect of Costus afer Ker Gawl aqueous leaf extract on lead-induced reproductive changes in male albino Wistar rats. JBRA assisted reproduction. 2019; 23(3): 215-224.
5. Alzahrani E, Alkhudidy AT. Synthesis, Optimization, and Characterization of Ecofriendly Production of Gold Nanoparticles Using Lemon Peel Extract. Int J Anal Chem : 2021; https://doi.org/10.1155/2021
6. Bhargavan D, Deepa B, Shetty H, Krishna, AP. The protective effect of Withania somnifera against oxidative damage caused by ethanol in the testes of adult male rats. IJBCP. 2015; 4(6): 1104-1108.
7. Jaâ M. A simple and practical method for rat epididymal sperm count (Rattus norvegicus). Biology Medicine and Natural Product Chemistry. 2015; 4(1): 1-3.
8. Trupti SP and Asmita AW. Review on therapeutic potential of ayurvedic medicine swarnabhasma (incinerated gold). 2018; IAJPS, 05 (01) : 192-205
9. Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, Saha AV, Mukherjee B. Evaluation of chemical constituents and free-radical scavenging activity of Swarnabhasma (gold ash), an Ayurvedic drug. J. Ethnopharmacol. 2002; 80(2-3):147-53.

10. Fazal Mohd, Vishnu Priya, Veeraraghavan, Bushra T, Selvaraj J, Gayathri R. Antioxidant effects of Emblica officinalis and Zingiber officinalis on arsenic and lead induced toxicity on Albino rats. Bioinformation. 2021;17(2): 295-305
11. Chang S, Jin B, Youn P, Park C, Park JD, Ryu DY. Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicol Appl Pharmacol. 2007; 218 (2):196-203.
12. Allouche L, Hamadouche M, Touabti A. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. Exp Toxicol Pathol. 2009; 61(5): 03-10.
13. Haitao L, Ruiyan N, Jinming W. Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. Research report fluoride. 2008; 41(3): 184-191.
14. Sainath SB, Meena R, Supriya CH, Reddy KP, Reddy PS. Protective role of Centella asiatica on lead-induced oxidative stress and suppressed reproductive health in male rats. Environ Toxicol Pharmacol. 2011;32(2):146-154.

HOW TO CITE THIS ARTICLE

Tripura M, Kamdi B, Ingole R, Ingawale M, Kuralkar P, Hajare S. Effect of Lemon peel extract mediated gold nanoparticles on hematobiochemical and sperm parameter alterations in lead- arsenic induced combined exposure in male rats. J Phytopharmacol 2024; 13(4):341-344. doi: 10.31254/phyto.2024.13411