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Research Article

THERAPEUTIC ASSESSMENT OF LEMON PEEL EXTRACT MEDIATED GOLD NANOPARTICLES AGAINST LEAD AND ARSENIC INDUCED REPRODUCTIVE TOXICITY IN MALE WISTAR RATS

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ABSTRACT: Gold has been used since ancient times as a rejuvenator, and reproduction enhancer in males and can be used for alleviating reproductive toxicity. Arsenic (As) and lead (Pb) exposure caused multi-organ toxicity, including reproductive organs. Therefore, a study was planned to assess the potential of lemon peel extract-mediated gold nanoparticles (LPGNP) on As and Pb-induced reproductive toxicity in male rats. The LPGNP synthesized with lemon peel extract and HAuCl4 and characterized by UV-VIS spectral analysis, FTIR spectrometry, XRD analysis, Zeta potential measurement, NTA and FESEM. Sodium Arsenate and Lead Acetate were administered to rats @13.8mg/kg and 116.4 mg/kg, respectively for 14 days followed by LPGNP supplementation at @10 and 20 mg/kg for 6 weeks. Alterations in reproductive parameters, antioxidant enzymes and histopathology of testes were recorded from different groups. In the results, GNP formation is indicated by changes from pale yellow to dark purple color formation in a combined solution of lemon peel extract and $HAuCl_4$. The peak absorbance at 557 nm confirms the synthesis of GNP. The LPGNP measures from 10 nm to 120 nm and the concentration was found to be 2.5 X 10^9 particles/ml. Our study showed serum testosterone concentration, sperm motility, total sperm count, sperm abnormalities, and sperm viability altered significantly in As and Pb toxic groups whereas LPGNP supplementation caused marked improvement. In histopathology of the testis, As and Pb caused degenerative changes of seminiferous tubules and sloughing of spermatogenic cells. These changes were observed as minimal in LPGNP-treated rats. Thus, LPGNP supplementation caused significant improvement against As and Pb-induced deleterious effects on the reproductive system.

Keywords: Arsenic, Lead, Lemon peel, Gold nanoparticles, Reproductive system.

INTRODUCTION

Since the time of Nagarjuna (800AD), gold has been used as a rejuvenator to enhance strength, and potency and to delay the aging process. In India, Ayurvedic physicians have been using gold ash (Swarnabhasma) as an aphrodisiac, to treat asthma, wasting of muscles, and mental disorders, and to enhance memory, immunity, vitality, and longevity [1]. Similar medical indications of gold can be found in many ancient medical classics. The lemon peel extract of citrus fruit [*Citrus limon* (Linn); Family: Rutaceae] is a rich source of flavonoids, glycosides, and coumarins; which may be exploited as reducing and capping agents in the synthesis of gold nanoparticles [2]. As and Pb are endocrine-disrupting, hazardous, non-essential heavy metals widely present in the environment and biota as pollutants. The Agency for Toxic Substances and Disease Registry has ranked arsenic as a top hazardous substance based on its toxicity, prevalence and potential for human exposure [3]. Major sources of arsenic exposure include contaminated food, water and agricultural and industrial effluents, with drinking contaminated water being the primary exposure route for populations in many regions worldwide [4]. The World Health Organization (WHO) has reported that the maximum permissible arsenic

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level in drinking water (10 µg/L) is frequently exceeded in several countries, putting approximately 100 million people at risk of exposure through drinking contaminated water [5, 6, 7]. Arsenic exposure leads to free radical generation, resulting in oxidative stress and disruption of macromolecules such as proteins, lipids, and DNA, potentially causing multi-system damage [3]. Arsenic exposure to the reproductive system causes a reduction in sperm count, and alterations in testicular and epididymal tissues histoarchitecture, therefore, testis and epididymis are considered prominent target sites of arsenic exposure [8]. Lead has a significant role in modern industry that bears a risk of occupational exposure from lead-contaminated dust, air, water, and food. It induces a broad range of physiobiochemical alterations and neurological dysfunctions that damage the liver, kidney, reproductive system and other physiological organs [9, 10, 11]. Numerous earlier reports pointed out that lead exposure has been correlated to altered sperm parameters which might further end up in male infertility [1, 12, 13]. Earlier literature indicated As and Pb exposure could be highly hazardous to the reproductive system which needs considerable attention, detailed toxicity studies, and therapeutic modalities to counter such adverse effects. Hence, it is imminent to find some remedial strategies to counter the As and Pb-instigated male reproductive toxicity.

Considering reproduction enhancing properties of gold, the present experiment was designed to evaluate the effect of green synthesized lemon peel extract-mediated gold nanoparticles on Pb and As induced subacute reproductive toxicity in male Wistar rats.

MATERIALS AND METHODS

Lemon peel extract mediated biosynthesis of gold nanoparticles

The biosynthesis of lemon peel extracts mediated gold nanoparticles was done as per the method of Alzahrani and Alkhudidy with slight modification [14]. The lemon peels from ripened citrus fruit were dried, and 20 g of lemon peel powder was put into 100 ml distilled water. The mixture then was kept at 80°C for 5 minutes, squeezed and filtered through muslin cloth and further through the Whatman No.1 filter paper. The extract thus obtained was used for the preparation of LPGNPs. The 2 mM of $HAuCl_4$ solution was added to 20% lemon peel extract solution in the ratio of 2:1 at room temperature (25 ± 3°C), with stirring and kept at a dark place. The color of the mixture indicates the formation of LPGNP.

Characterization of LPGNP

The characterization of LPGNP was carried out through techniques such as UV visible spectrum analysis, Nanoparticle Tracking Analyser (NTA), Field Emission Scanning Electron Microscopy (FESEM), Zeta Potential measurements, X-ray diffraction analysis (XRD), and Fourier-transform Infrared Spectroscopy (FTIR). These tests were performed in the Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati (MS, India).

Chemical treatment

Sodium arsenate ($NaAsO_2$) and Lead acetate ($C_4H_6O_4Pb$) used for induction of subacute toxicity were of analytical grade procured from Himedia Ltd. India. Freshly prepared stock solution of As and Pb in distilled water was used for administration to experimental animals via oral intubation.

In vivo experimental protocol

The forty-two healthy adult male Wistar rats of 160 to 190 g were acclimatized for a week before the start of the experiment in the laboratory of the Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola (M.S.) under standard managemental conditions. The Institutional Animal Ethics Committee approved all the experimental protocols (Approval No. IAEC/312/2000/02/2022). The experimental animals were housed in polypropylene cages and were provided with rat pellet chow (Nutrivet Life Sciences, Pune, India) and water ad-libitum. The animals were maintained under a 12-12 h cycle of darkness the temperature was maintained at 25±2°C and the relative humidity was 30-50% during the experiment. The rats were randomly divided into seven groups and received Sodium Arsenate and Lead acetate @13.8mg/kg and 116.4 mg/kg, p.o. respectively for 14 days followed by LPGNP supplementation @10 and 20 mg/kg for 6 weeks (Table 1). The body weight of animals was recorded weekly till the end of the experiment.

Testosterone assay

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

In brief, antibody-coated tubes were labeled in duplicate for testosterone standards, controls, and

unknown serum samples. Then, 50 µL of each standard, control, and unknown serum sample was pipetted into the appropriate tubes. A 300 µL I125-labeled testosterone tracer was added to each tube and mixed by vortexing. All tubes were covered and incubated for 1 hour at 18-25°C with shaking at ≥ 400 rpm. Subsequently, tubes were decanted and washed with 2 ml of wash solution and the radioactivity was measured for one minute with the help of a gamma counter.

Sperm parameters analysis

The total spermatozoa count was performed as per the standard method [15]. Briefly, 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). The mixtures were then incubated for 30 min at room temperature. A total of 10 µl of sperm suspension was taken from the 10 ml sample and then charged on the hemocytometer from both sides. Then hemocytometer was allowed to settle the sperm on a counting grid. Counting 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. The abnormal spermatozoa count and sperm motility were performed as per the methodology described by Bhargavan [16]. A total of 200 sperms were counted. Sperm motility was evaluated within 2-3 minutes from prewarmed 0.1 M phosphate-buffered saline (pH 7.2) and minced cauda epididymal mixed fluid and observations were recorded under a compound microscope. For abnormal spermatozoa count 5-6 drops of Eosin-Nigrosin stain was used and checked for sperm viability. Minced epididymal fluid in 0.1 M phosphate-buffered saline (pH 7.2) mixed with Eosin-Nigrosin stains and slides were prepared, dried, and then observed under a compound microscope for sperm abnormality.

Superoxide dismutase (SOD) analysis

SOD activity was assayed following the standard method [17]. To prepare the sample, 1 ml of testicular tissue homogenate was combined with 1.25 ml of chloroform and 0.25 ml of ethanol and then shaken mechanically for 15 minutes. The mixture was centrifuged at 20,000 x g for 15 minutes. Subsequently, 0.5 mL of the supernatant was added to 2.0 mL of 0.1 M Tris-HCl buffer (pH 8.2), 1.5 mL of distilled water, and 0.5 mL of pyrogallol. Optical density was measured at 420 nm at intervals of 0, 1, and 3 minutes. A similar procedure was followed for control tubes using 0.5 mL of distilled water against a buffer blank. Enzyme activity was expressed as Units/mg protein, with one unit (U) defined as the amount of enzyme needed for 50% inhibition of pyrogallol auto-oxidation. Absorbance increase at 420 nm was recorded over three minutes using a spectrophotometer. The following equations were used to calculate enzyme units:

% of inhibition = [(A-B) *100]/B.
Enzyme unit (U) = (% of inhibition/50)
*Common dilution factor (100).

Where 50% inhibition is similar to 1 U; A-control sample and B-test sample.

Glutathione reductase (GR) analysis

GR activity was determined using Ellman's reagent [18]. For this, 10 mg of reduced glutathione was dissolved in 100 mL of distilled water. Testicular tissue (1 g) was homogenized in 0.2 M phosphate buffer (pH 8). From this homogenate, 0.5 mL was pipetted and precipitated with 2 mL of 5% trichloroacetic acid (TCA). After centrifugation, 1.5 mL of the supernatant was collected, to which 1 mL of Ellman's reagent and 3 mL of phosphate buffer were added. Once the reaction was complete, absorbance was measured at 412 nm against a PBS and supernatant blank. A standard curve was generated using known GR concentrations, and the sample absorbance values were compared accordingly.

The concentration of an enzyme can be calculated using the formula:

$$\text{Units/ml} = \frac{(\blacktriangle A \text{ sample} - \blacktriangle A \text{ blank}) \text{ X (dilution factor)}}{\varepsilon nm \text{ X (Volume of sample in ml)}}$$

For DTNB εnm (Ellman's reagent)=14.15 Mm^{-1} cm^{-1}.

Catalase (CAT) analysis

Catalase activity was analyzed using a standard procedure [19]. For this assay, 0.1 mL of testicular tissue homogenate was combined with 0.5 mL of H_2O_2 and 1.0 mL of buffer. The reaction start time was noted, followed by the addition of 2.0 mL of dichromate-acetic acid. The chromophore compound formed was measured at 570 nm. Catalase activity was calculated using a molar extinction coefficient of 43.6 cm^{-1}, expressed as mmol of H_2O_2 decomposed per minute per mg of protein as (ΔA/min x 1000 x 3)/(43.6)/mg protein in sample).

Histopathological examination

Tissues of testes from different groups were collected in 10% formal saline solution for histopathological examination after sacrificing rats using thiopental sodium as anesthesia. After fixation, tissues were processed using alcohol and xylene followed by impregnation in paraffin wax (Qualigen) as per routine method and sections of 5µ were cut and stained with H & E stain as per the method described by Luna [20] for recording histopathological observations.

Statistical analysis

The data were expressed as mean ± standard error. The data were statistically analyzed by one-way analysis of variance (ANOVA) with treatment as a factor and the means were compared by Tukey's test as post hoc analysis in IBM SPSS Statistics, Version 22 software. $p<0.05$ was considered significant.

Table 1. Different treatment groups, dose and route of administration.

Group	Treatment, Dose / route(Oral)	No. of rats
I	Control – DW for 8 weeks	6
II	Sodium Arsenite 13.8mg/kg BWt for 14 days	6
III	Lead acetate 116.4 mg/kg BWt for 14 days	6
IV	Sodium Arsenite 13.8mg/kg for 14 days and LPGNP 10mg/kg from 15th day till last day of experiment	6
V	Sodium Arsenite 13.8mg/kg for 14 days and LPGNP 20mg/kg from 15th day till last day of experiment	6
VI	Lead Acetate 116.4 mg/kg for 14 days and LPGNP 10mg/kg from 15th day till last day of experiment	6
VII	Lead Acetate 116.4 mg/kg for 14 days and LPGNP 20mg/kg from 15th day till last day of experiment	6

Total experimental period was 8 weeks.

RESULTS AND DISCUSSION

In the Indian traditional and Ayurvedic system of medicine, gold in the form of gold ash (bhasma) is used in some disease conditions like psychosomatics disorders, arthritis, and loss of vigor and vitality [21]. Gold has been thought to be safer than other heavy metals to be used in therapeutics at the nanoscale due to its resistance to oxidation. In the present study, it was found that biosynthesized LPGNP @10 and 20 mg/kg administered to rats for six weeks caused no adverse effects. The doses of LPGNP selected in the present study at 10 and 20 mg/kg are far below the level of toxicity study of the previous report indicating that gold nanoparticles showed no evidence of toxicity in acute, sub-acute, and chronic toxicity studies conducted in mice [1]. Worldwide toxic metals exposure remains a widespread occupational and environmental problem. Heavy metals toxicants, As and Pb generally interfere with CNS, hematopoietic, hepatic, renal and reproductive functions. Exposure to As and Pb caused oxidative stress, neurological diseases, and reduced testosterone levels. As per Ayurveda, ancient literature, and earlier reports gold can be used to enhance testosterone levels and some reproductive functions in males [22, 23]. Considering the medicinal properties of gold, the present study has been designed to evaluate the effect of green

Table 2. Mean weekly body weight gain in control and different treatment group of rats.

Group	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week
I	4.83±0.94^{a}	22.33±2.82^{a}	13.16±1.66	15.5±1.5	14.76±1.25	17.0±1.6	16.0±0.44	16.16±1.47
II	1.16±0.16^{c}	16.16±1.3^{bc}	14.66±1.76	12.33±1.05	14.66±2.95	14.5±1.76	14.16±2.22	13.5± 1.5
III	1.33±0.21^{c}	15.83±1.4^{bc}	11.5±1.47	14.0 ±1.06	13.16±0.79	13.0±0.73	13.16±0.94	14.33±0.61
IV	3.16±0.6^{ab}	20.0±0.68^{ab}	13.55±0.99	15.16±1.19	14.0±1.80	16.16±1.9	15.83±1.24	15.5±1.08
V	4.16±0.70^{a}	20.66±2.66^{ab}	13.5±1.72	15.33±1.25	14.16±0.87	15.33±0.91	15.16±1.77	14.66±1.58
VI	4.0±0.44^{a}	20.33±0.66^{ab}	13.83±0.83	14.5±1.20	14.0±1.00	16.0±0.73	15.0±1.31	14.83±0.87
VII	4.83±0.47^{a}	20.83±0.94^{ab}	14.83±1.57	14.16±1.60	14.17±1.86	16.5±0.99	15.16±0.79	15.33±1.52
*Sign/NS CD(0.05)	1.740	5.204	NS	NS	NS	NS	NS	NS

Values indicate mean ± S.E. Data was analyzed using one way ANOVA followed by Tukey's post hoc test in IBM SPSS software, Version 22. Mean bearing different superscripts differ significantly. *Significance level $p<0.05$, NS - Non significant.

Table 3. Effect of lemon peels extracts gold nano particles in subacute toxicity of lead and arsenic 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^{e}	83.67 ±1.72^{a}	3.13 ± 0.24^{a}
II	48.83 ±2.62^{d}	102.73 ±2.41^{f}	16.66 ± 1.20^{a}	55.50±2.90^{cd}	0.83 ± 0.15^{d}
III	52.66±2.10^{cd}	135.83 ± 5.47^{e}	16.50 ± 0.76^{a}	50.33± 1.06^{d}	1.13 ± 0.11^{d}
IV	61.00±2.43^{ab}	141.63 ± 3.60^{de}	8.50 ± 0.56^{b}	64.00±1.80^{b}	1.28 ± 0.15^{d}
V	59.50±2.81^{ab}	132.33 ±9.85^{e}	6.50 ± 0.67^{cd}	61.16±2.60^{bc}	2.39 ± 0.18^{bc}
VI	55.83±2.86^{bc}	166.53 ± 4.93^{cd}	9.16 ±0.70^{b}	63.50± 1.31^{b}	2.27 ± 0.23^{c}
VII	63.00± 2.25^{a}	198.83 ± 7.01^{ab}	6.33 ±0.61^{cd}	63.83± 2.49^{b}	2.39 ±0.31^{bc}
*Sign/NS CD(0.05)	6.780	28.73	2.377	5.920	0.581

Values indicate mean ± S.E. Mean bearing different superscripts differ significantly. *Significance level p<0.05, NS - Non significant.

Table 4. Testes SOD, CAT and Glutathione reductase in control and different treatment group of rats.

Groups	Superoxide dismutase	Catalase	Glutathione reductase
I	2.15 ± 0.15^{a}	47.18 ± 2.64^{a}	1.39 ±0.08^{a}
II	1.25 ± 0.10^{de}	21.14 ± 2.53^{e}	0.73 ± 0.04^{bcd}
III	1.34 ±0.07^{cde}	24.61 ± 2.09^{cde}	0.64 ± 0.05^{cd}
IV	1.39 ± 0.10^{cd}	26.47 ± 2.10^{bcde}	0.68 ± 0.02^{cd}
V	1.32 ± 0.08^{cde}	31.36 ± 1.28^{b}	0.61 ± 0.03^{d}
VI	1.47 ± 0.06^{cd}	26.59 ± 2.63^{bcde}	0.74 ± 0.04^{bc}
VII	1.54 ±0.05^{bc}	27.69 ± 1.33^{bcd}	0.76 ± 0.05^{bc}
*Sign/NS CD(0.05)	0.253 (0.05)	5.527 (0.05)	0.134 (0.05)

Values indicate mean ± S.E. Mean bearing different superscripts differ significantly. *Significance level p<0.05, NS - Non Significant.

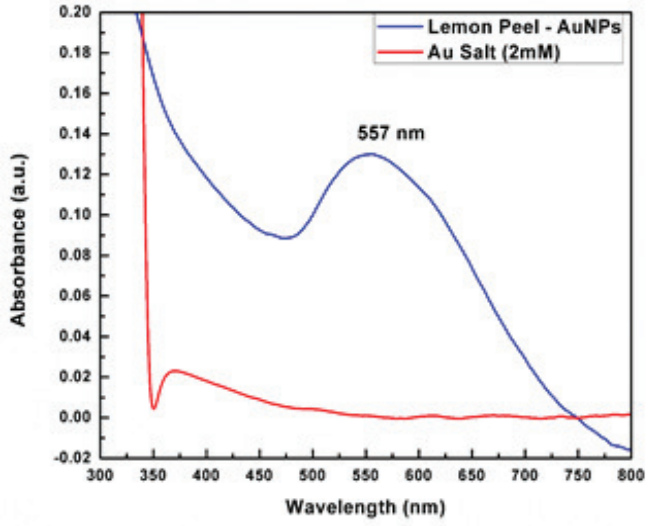

Fig 1. UV-VIS spectral analysis.

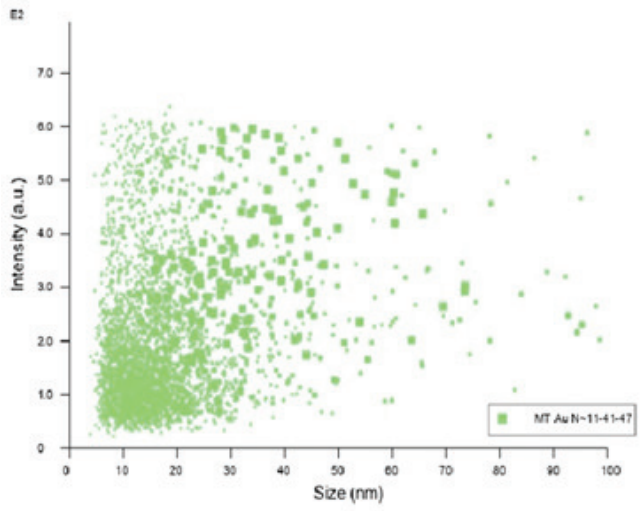

Fig. 2. Nanoparticles tracking analysis.

synthesized LPGNPs against As and Pb-induced subacute reproductive toxicity in male rats.

When a gold chloride solution is continuously stirred with lemon peel extract, the $HAuCl_4$ solution undergoes rapid reduction, resulting in a color change from pale yellow to ruby red. This distinct color change is likely due to the excitation of surface plasmon vibrations in the gold nanoparticles. As per Mie theory, small, spherical gold nanoparticles exhibit a single surface plasmon resonance (SPR) absorption

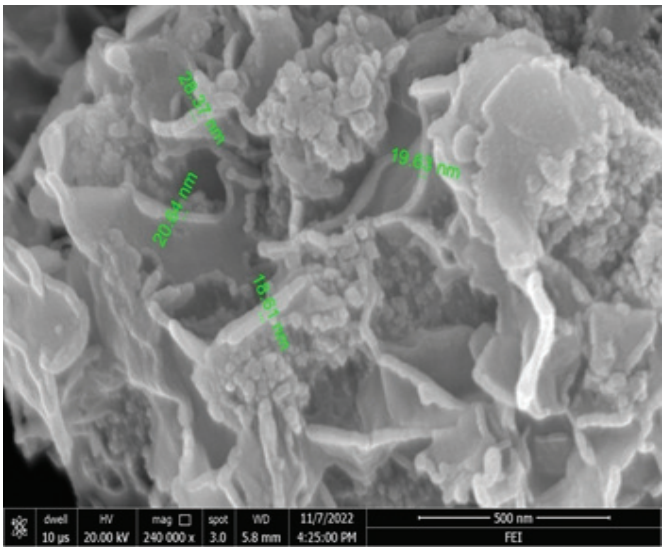

Fig. 3. Field emission scanning electron microscopy image of LPGNPs .

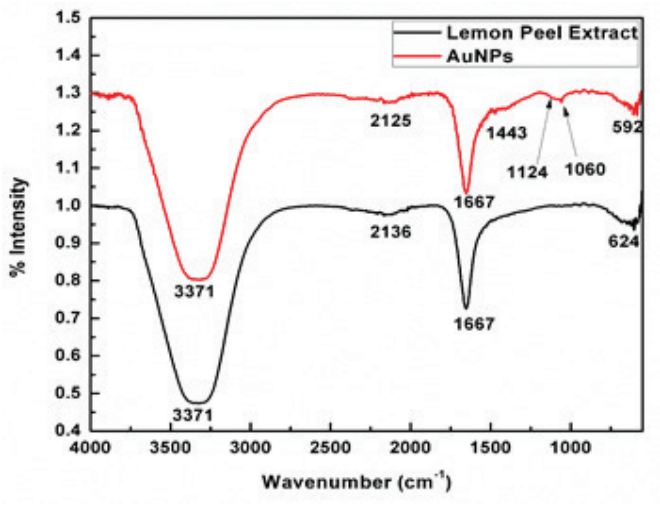

Fig. 4. FTIR (Fourier transform infrared) spectroscopy.

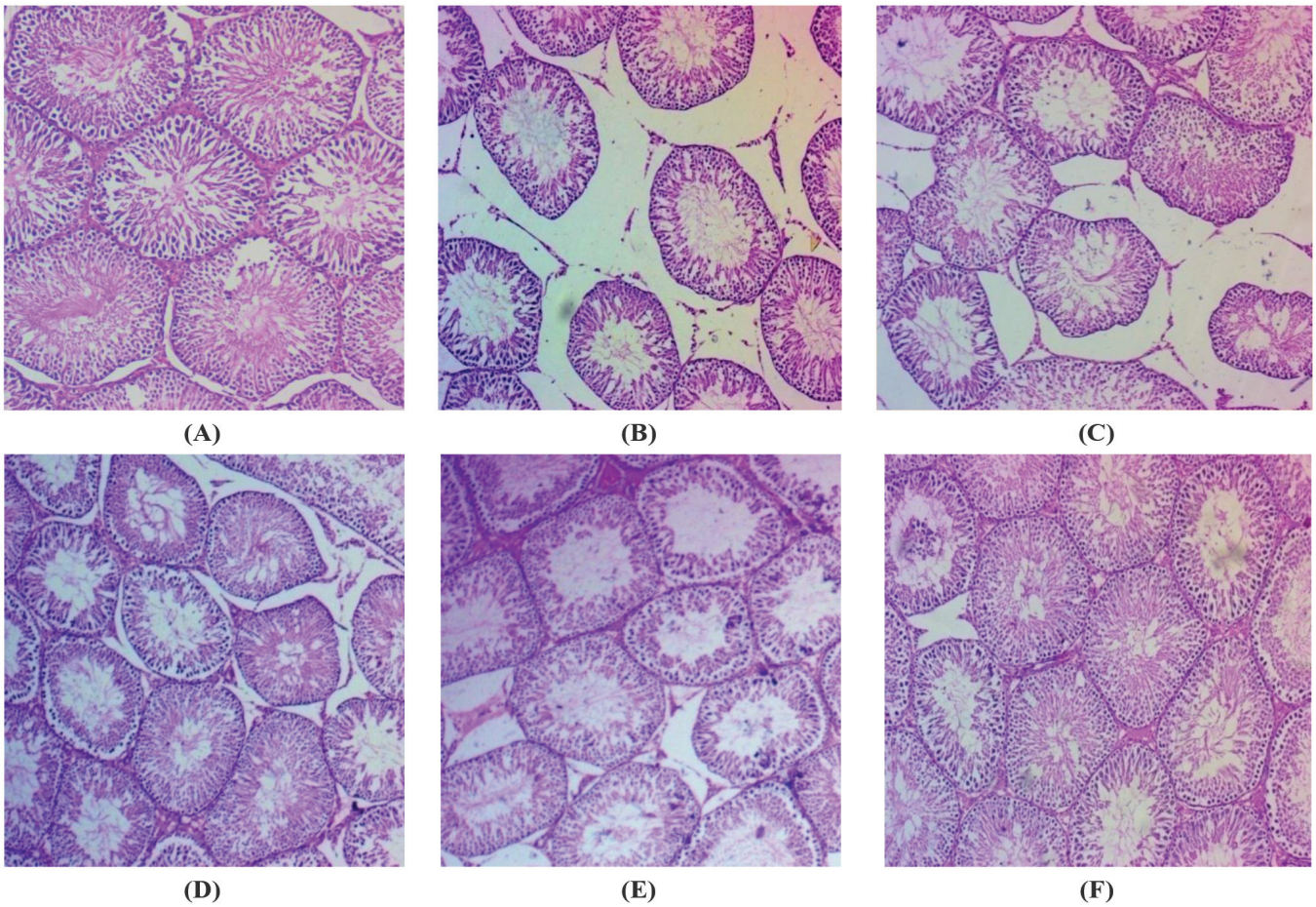

Fig. 5. Microphotograph of testes of rat. (A): Control group; (B): 13.8 mg/kg body weight arsenic treated for 14 days; (C):116.4 mg/kg body weight lead treated group for 14 days; (D): arsenic (13.8 mg/kg body weight) treatment for 14 days + LPGNP (10 mg/kg body weight) treatment for 6 weeks started from 15th day till end of experiment; (E): arsenic (13.8 mg/kg body weight) treatment for 14 days + LPGNP (20mg/kg body weight) treatment for 6 weeks started from 15th day till end of experiment; (F): lead (116.4 mg/kg body wt.) treatment for 14 days + LPGNP (20mg/kg body weight) treatment for 6 weeks started from 15th day till end of experiment.

band, whereas anisotropic particles display two or more SPR bands [2]. The UV-vis spectra showed peak absorbance at 557 nm which is characteristic of gold nanoparticles (Fig. 1). The surface plasmon resonance observed in the range of 535-580 nm corresponds with the findings of Alzahrani and Alkhudidy [14] who synthesized gold nanoparticles from lemon peel extract. NTA analysis confirmed the synthesis of nano sized GNPs with an average size of 109 nm (Fig. 2). The size was further confirmed by FESEM (Fig. 3). The distribution of GNPs observed in two dimensions when plotted as average nanoparticle size against the % intensity of the nanoparticles. The distribution graph indicated that most nanoparticles ranged between 20 and 120 nm, with some variation in their average size. The concentration of GNPs was determined to be 2.5 x 10^9 particles/ml. Nanoparticle Tracking Analysis (NTA) estimates particle size using the Stokes-Einstein equation, which relates to the size of individual particles. By analyzing size and intensity, NTA allows for microscopic visualization of suspended nanoparticles, enabling the tracking of each particle's Brownian motion to characterize the particle population [24]. LPGNPs were observed in the range of 20 nm to 120 nm which corroborates with the reports of Arancon *et al.* and Alzahrani and Alkhudidy [14, 25]. The synthesized gold nanoparticles were coated with metabolic compounds from lemon peel extract, and their stability was assessed by zeta potential measurement. The zeta potential of LPGNPs was found to average -19.7, indicating moderate stability. X-ray diffraction (XRD) analysis confirmed the crystallinity and phase purity of the gold nanoparticles, matching the JCPDS 04-0783 standard for gold, consistent with recent findings [26]. The XRD spectrum of AuNPs displayed distinct diffraction peaks at (111), (200), (220), and (311) planes within the 2θ range of 30°-90°. The Fourier transform infrared (FTIR) spectrum showed characteristic bands between 1667 and 624 cm^{-1} (Fig. 4). Previous research has also documented the successful synthesis of stable gold nanoparticles using various lemon varieties and extracts [14, 27, 28].

The rats in As and Pb toxic control groups showed mild aggressiveness, stress, and mild anorexia during the initial weeks (up to 3rd week) of the experiment which is reflected by a significant ($p<0.05$) decrease in body weight gain in these animals. However, LPGNP-treated animals did not show abnormal behavior or clinical signs, and the body weight gains in these groups were comparable to normal control but differed significantly from toxic control up to 3rd week. A further nonsignificant difference in weight gain was observed from the 4th week to the 8th week in all treatment groups including normal and toxic controls (Table 2).

The sperm motility and total sperm count in the As and Pb toxic control group decreased significantly ($p<0.05$) as compared to the control group. In the comparable toxicity, arsenic caused more decrease in sperm count than lead. In As + LPGNPs and Pb + LPGNPs treatment groups sperm motility and total sperm count were found to improve significantly ($p<0.05$) as compared to toxic controls. A significant dose-dependent recovery in total sperm count was observed in all LPGNP treatment groups that received As and Pb (Table 3). In reproductive parameters, As and Pb exposure resulted in significantly reduced total sperm count, motility, viability of sperms, and increased sperm abnormalities which coincides with findings of earlier reports [30, 31]. Our findings on lead toxicity on different sperm parameters align with the study of Allouche *et al.* [32] revealed that the percentage of total motility was decreased in rats given lead acetate and total sperm abnormalities were elevated in all lead-exposed groups as compared to control. Both As and Pb decreased serum testosterone levels significantly ($p<0.05$) in toxic control groups but As caused severe depletion of testosterone levels (group II) which may lead to inhibition of spermatogenesis and sperm maturation. Past studies revealed that As binds with thiol groups of biological molecules, resulting in detrimental effects on the male reproductive system such as a reduction in the testosterone level [33]. The earlier report suggested that exposure to Pb may affect sperm parameters, elevation in morphological abnormalities, and sperm DNA integrity [30]. The administration of LPGNPs in As and Pb intoxicated groups caused significant ($p<0.05$) and dose-dependent revival in serum testosterone as compared to toxic control groups.

The SOD values of testes showed significant ($p<0.05$) differences between control and different treatment groups. The SOD depletion was observed higher in arsenic toxic group than in lead toxic group. The marginal improvement in SOD was observed only in LPGNP+Pb treated groups which indicates that LPGNP administration has no significant revival effect on SOD depletion caused by As and Pb. The CAT and GR levels depleted significantly ($p<0.05$) in all treatment groups including toxic groups as compared to control. Mild improvement was observed in CAT and GR enzyme levels in different LPGNP-treated

groups as compared to toxic controls (Table 4). Free radicals are commonly generated by auto-oxidation which leads to oxidative stress. The cellular antioxidant system scavenges free radicals that deplete antioxidant enzymes; which is a major cause of the toxic effects of heavy metals [34]. Numerous studies pointed out that the toxicity occurring due to As exposure is due to a reduction in anti-oxidative enzyme activity [35]. Pb is shown to inhibit functional SH groups in several enzymes such as ALAD, SOD, and CAT and thereby alters the antioxidant activities [36]. Thus the current findings on antioxidant enzymes are consonant with several previous studies.

Sections of the testis from rats of the control group showed well-organized basement membrane with well-ordered leydig cells and sertoli cells, a lumen packed with spermatids, and normal spermatozoa with an attached tail (Fig. 5A). In treated rats, testis sections showed increased intertubular spaces and reduction in the size of tubules, degenerative changes of seminiferous tubules, widening of interstitial spaces, derangement, and sloughing of spermatogenic cells (Fig. 5B). In the lead acetate-treated group of rats, testis sections revealed detachment of germinal cells from the basement of the tubule, increased tubular space, and mild necrotic and degenerative changes in the seminiferous tubule (Fig. 5C). Sections from As + LPGNPs group showed comparatively reduced intertubular spaces, and degenerative changes and reduction the size of the tubule (Fig. 5D and 5E). Pb + LPGNPs Group revealed edema and mild detachment of germinal cells from the basement membrane (Fig 5F). The histopathological findings in this study align with previous reports indicating that As and Pb exposure lead to testicular histological changes, causing structural abnormalities in rat spermatids and sperm. Solaimani and coworkers observed that arsenic-treated male rats displayed vacuolar degeneration in the spermatogenic epithelium, thickened basement membranes, and necrosis of Leydig cells [37]. Similarly, Liu and coworkers reported that male rats exposed to lead acetate showed disrupted germ cell arrangement and a reduced spermatogenic cell layer in the seminiferous tubules [38].

CONCLUSION

The result of the present study concludes that Pb and As exposure caused significant alterations in reproductive parameters and antioxidant enzymes. Upon LPGNP oral administration @ 10 and 20mg/kg marked to moderate improvement was observed in sperm parameters, testosterone levels, and histomorphology of testis in As and Pb toxicity. These findings supported Ayurvedic and traditional use of gold as a rejuvenator and enhancer of male sexual function. However, further broader research on gold nanoparticles is required before its possible application in male reproductive disorders and also more detailed molecular studies are needed for their safety analysis.

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REFERENCES

1. Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, *et al.* Evaluation of chemical constituents and free-radical scavenging activity of Swarnabhasma (gold ash), an ayurvedic drug. J Ethnopharmacol. 2002; 80(2-3):147-153.

2. Shahnah SM, Ali S, Ansari H, Bagri P. New sequiterpene derivative from fruit peel of *Citrus limon* (Linn) Burn F Sci Pharm. 2007; 75:165-170.

3. Rahaman MS, Rahman MM, Mise N, Sikder MT, Ichihara G, *et al.* Environmental arsenic exposure and its contribution to human diseases, toxicity mechanism and management. Environ Pollut. 2021; 289:117940.

4. Vergara-Gerónimo CA, Del Río AL, Rodríguez-Dorantes M, Ostrosky-Wegman P, Salazar AM. Arsenic-protein interactions as a mechanism of arsenic toxicity. Toxicol Appl Pharmacol. 2021; 431:115738.

5. Missimer TM, Maliva RG. Environmental issues in seawater reverse osmosis desalination: Intakes and outfalls. Desalination. 2018; 434:198-215.

6. Kumar A, Rahman MS, Ali M, Salaun P, Gourain A, *et al*. Assessment of disease burden in the arsenic exposed population of Chapar village of Samastipur district, Bihar, India, and related mitigation initiative. Environ Sci Pollut Res. 2022; 1:1-7.

7. Nunes LM, Otero X. Quantification of health risks in Ecuadorian population due to dietary ingestion of arsenic in rice. Environ Sci Pollut Res. 2017; 24:27457-68.

8. Souza AC, Marchesi SC, Lima DAG, Ferraz RP, Santos FC, *et al.* Effects of sodium arsenite and arsenate in testicular histomorphometry and antioxidants enzymes activities in rats. Biol Trace Elem Res. 2016; 171:354-362, DOI:10.1007/s12011-015-0523-0.

9. Ezejiofor AN, Orisakwe OE. The protective effect of *Costus afer* Ker Gawl aqueous leaf extract on lead-induced reproductive changes in male albino Wistar rats. JBRA Assist Reprod. 2019; 23(3):215.

10. Dapul H, Laraque D. Lead poisoning in children. Adv Pediatr. 2014; 61(1):313-333.

11. Kumar SB, Dada R, Gupta NP. Environmental toxicants-induced male reproductive toxicity: role of oxidative stress. In:

Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. 2018; 305-322, Academic Press.

12. Wahab OA, Princely AC, Oluwadamilare AA, Oore-oluwapo DO, Blessing AO, Alfred EF. Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. JBRA Assist Reprod. 2019; 23(4):336.

13. Needleman H. Lead poisoning. Annu Rev Med. 2004; 55:209-222.

14. Alzahrani E, Alkhudidy AT. Synthesis, optimization, and characterization of eco-friendly production of gold nanoparticles using lemon peel extract. Int J Anal Chem. 2021, DOI:10.1155/ 2021.

15. Khanavi M, Hadjiakhoondi A, Roodsari HR, Vosoughi M, Arbabi R. The effects of ethanolic extracts of *Melia indica* and *Melia azedarach* fruits on reproductive indices of male rats. J Reprod Infertil. 2007; 8(1):7-16.

16. Bhargavan D, Deepa B, Shetty H, Krishna A. The protective effect of *Withania somnifera* against oxidative damage caused by ethanol in the testes of adult male rats. Int J Basic Clin Pharmacol. 2015; 4(6):1104-1108.

17. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3):469-474.

18. Owens CW, Belcher RV. A colorimetric micro-method for the determination of glutathione. Biochem J. 1965; 94(3):705.

19. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47(2):389-394.

20. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. In: Manual of histologic staining methods of the Armed Forces Institute of Pathology. 1968.

21.Trupti SP, Asmita AW. Review on therapeutic potential of ayurvedic medicine swarnabhasma (incinerated gold). 2018; Indo Americ J Pharmaceutic Sci. 5(01):192-205.

22. Semmler-Behnke M, Lipka J, Wenk A, Hirn S, Schäffler M, *et al*. Size dependent translocation and fetal accumulation of gold nanoparticles from maternal blood in the rat. Part Fibre Toxicol. 2014; 11:1-2.

23. Sharma H, Rasayogasagar II, 2nd edn., 1983; Krishnadas Academy, Choubhambha Press, Varanasi, India, 529-530.

24. Pechyen C, Ponsanti K, Tangnorawich B, Ngernyuang N. Waste fruit peel-Mediated green synthesis of biocompatible gold nanoparticles. J Mate Res Technol. 2021; 14:2982-2991.

25. Arancon RA, Lin D, Chen SH, Lin G, Lai CSK, *et al.* Nanoparticle tracking analysis of gold nanomaterials stabilized by various capping agents. RSC Advances. 2014; 4(33): 17114-17119.

26. Sivakavinesan M, Vanaja M, Lateef R, Alhadlaq HA, Mohan R, *et al. Citrus limetta* Risso peel mediated green synthesis of gold nanoparticles and its antioxidant and catalytic activity. J King Saud Univ Sci. 2022; 34(7):102235.

27. Elavazhagan T, Arunachalam KD. Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. Int J Nanomed. 201;1265-1278.

28. Pandey S, Goswami GK, Nanda KK. Green synthesis of polysaccharide/gold nanoparticle nanocomposite: an efficient ammonia sensor. Carbohydr Polym. 2013; 94(1):229-234.

29. Wani IA, Ahmad T. Size and shape dependant antifungal activity of gold nanoparticles: a case study of Candida. Colloids Surf B: Biointerfaces. 2013; 101:162-170.

30. 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.

31. Jana K, Jana S, Samanta PK. Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: possible an estrogenic mode of action. Reprod Biol Endocrinol. 2006; 4:1-3.

32. 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):503-510.

33. Mehrzadi S, Bahrami N, Mehrabani M, Motevalian M, Mansouri E, Goudarzi M. Ellagic acid: A promising protective remedy against testicular toxicity induced by arsenic. Biomed Pharmacother. 2018; 103:1464-1472.

34. Tyagi S, Kalia K, Chundawat R, Sood P. Heavy metal intoxication, oxidative stress and antioxidants therapy: a review. J Cell Tissue Res. 2018; 18(3):6583-6611.

35. Mahajan L, Verma PK, Raina R, Sood S. Toxic effects of imidacloprid combined with arsenic: Oxidative stress in rat liver. Toxicol Ind Health. 2018; 34(10):726-735.

36. Vallee BL, Ulmer DD. Biochemical effects of mercury, cadmium, and lead. Annu Rev Biochem. 1972; 41(1):91-128.

37. Solaimani F, Habibi E, Ghasemi M, Mahboubi S, Zamani E, Shaki F. The protective effects of *Trametes versicolor* on arsenic-induced male reproductive toxicity through regulation of oxidative stress: a biochemical and histopathological survey. Andrologia. 2023; 2023(1):7579366.

38. Liu HT, Niu RY, Wang JM, He Y, Wang JD, China S. Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. Fluoride. 2008; 41(3):184-191.

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