CHAPTER-5

NANOTOXICOLOGY OF SILVER AND GOLD NANOPARTICLES

Introduction

There is immense growth in nanotechnology field during last few years which facilitated innovative research and in industrial production of nanoparticles, which has raised questions about their potential toxicity [1]. Very recently the concept of nanotechnology is effectively used as a tool for the diagnosis and treatment of various life-threatening diseases [2]. The interest on the study of toxicity aspects is in vogue since from history, like, metal fumes exposure effect in humans. But, only recently the some serious effort is being made to understand the potential toxicity of these particles. In this advanced and applied research work we have made a sincere effort in noting the efficacy and safety of the functionalized noble nanoparticles *in-vivo*. The noble metal nanoparticle synthesis, particularly AgNP and AuNP are easy to synthesize the particles of different shapes and sizes which can be functionalized using different biological moieties like flavonoids, proteins and sugars for their possible pharmacological applications [3]. We have developed these functionalized nanoparticles with an objective to use as drug delivery system for the future applications of advanced diagnosis and treatment of different life-threatening diseases. Before conducting the clinical studies of these materials it is important to understand the nanotoxicology profile.

In this research we have reported the studies conducted on the safety parameters of functionalized noble metals using different bioorganic moieties of plants. These functionalized AgNP and AuNP are administered to Sprague-Dawley rats for 28 days in three different doses. These studies reveal that both these functionalized nanoparticles can be safely used *in vivo* up to the dose range of 500 mg/kg body wt. Though there are incidences like hepatic focal necrosis and vacuolization observed; they are unrelated to the prescribed treatment up to higher concentration as the same effect is also observed in untreated group. AgNP are already popular because of their good number of applications in water purification systems, dental implants and making of toothpastes, cosmetic preparations including shampoo manufacturing, nursing aids and preparations of nipples, air-fresheners, filtering aids, domestic aids, sports materials etc. [4, 5]. Normally inner part of the all the above aids are coated with AgNP as it is lethal bactericidal. The medical aids like surgeon mask and hand glows are also coated with AgNP [6] and also AgNP is used in the treatment of burns and wounds [7, 8].

U.S. Agency for Toxic Substances and Disease Registry (ATSDR, 2005) have published a review note on the noxious effects of different forms of silver like silver sulfide, silver chloride, silver oxide stating that the effect of these on the human health requires long time and short time extensive studies [9]. In another report published by U.S. Environmental Protection Agency (U.S. EPA) suggests that in drinking

water, the permitted level of Ag to be <50 μg/L, for short-term exposure (1–10 days).

Considering all the above warnings we have made an effort to study the safety profile of these functionalized nanoparticles *in vivo* on Sprague-Dawley rats. These animals were administered intravenously by functionalized AgNP as per the standard test guidelines 407 of Organization for Economic Cooperation and Development (OECD). The guideline explains the procedure of repeat-dose toxicity study for 28 days.

The study infers that these functionalized noble metal nanoparticles can be safely used *in vivo* up to the concentration of 500 μg/ kg body wt. Up to this concentration there is no treatment related microscopical, biochemical, histopathalogical abnormalities are seen. There is no effect on the morphology of tissues, serum analysis, ophthalmic activity and behavior changes up to the highest dose of administration inferring that there is no mortality related issues with the administration of these nanoparticles.

5.1 Materials and Methods

5.1.1 Animals and Housing

Sprague Dawley rats – Hsd: SD rats conventionally bred (In-house random bred), aged 8 weeks, males weighing 240-290 g and females weighing 160-220 g were used for the experiment. Rats housed with fresh air in an air-conditioned environment under standard laboratory conditions (19 -24 °C, RH - 30 - 70 %). Two rats per cage were housed in standard suspended polysulfone cages (425

x 266 x 175 mm size) each having grill facilities and top of stainless steel with a facility for holding pelleted food and drinking water in polycarbonate bottles. Paddy husk was used as bedding material. The cage along with the paddy husk was changed at least twice a week. The rats were fed rodent pelleted diet (Hindustan Lever Limited, Bombay) and filtered water *ad libitum*.

5.1.2 Vehicle and Dose Selection

Three dosage concentrations consisting 10 µg/kg, 200 µg/kg and 500 µg/kg, Bwt/day were selected based on the preliminary study conducted at our laboratory. The test item is soluble in distilled water; the same is selected as a vehicle.

5.1.3 Treatment and Observations

The test solution of silver and gold nanoparticles solution was prepared in distilled water for complete dissolution and was administered by intravenous route to the experimental rats. The control animals administered an equivalent amount of injectable water through intravenous route.

The test item of silver and gold nanoparticles solution and the control were administered to rats of respective groups for the specific treatment once daily at the same time each day (varied by ±2 h) for 28 consecutive days. This study was conducted according to OECD 407 guideline [1].

General symptoms of clinical signs are noted everyday, before administering the dose, after ~2 h. Rats were observed daily for any changes in fur and skin, posture and response to handling, mucous

membrane, autonomic activity, eyes, occurrence of secretions excretions, and changes in gait.

Ophthalmological examination for toxicity group rats was conducted with an ophthalmoscope before start of the treatment and prior to sacrifice, at the end of dosing. Mydriasis was induced before examination using a 1 % solution of Tropicamide.

5.1.4 Food Intake and Body Weights

Ref:

Tab. 5.1, 5.2 and **5.3** for AgNP

Tab. 5.8, 5.9 and **5.10** for AuNP

Food consumption was calculated by using the food consumed at each measuring interval per cage and divided by no. of rats in each cage per day in the intervening period to determine the food intake/rat/day.

Body weights of each rat were noted before the administration of nanoparticles on days 1, 8, 15, 22 and 28 of the dosing period. Fasting body weight was recorded prior to sacrifice on 29th day.

5.2 Clinical Pathology Investigations for Toxicity Groups

All animals were kept on fast for one night of 27th day. Before the treatment on 28th day) the blood was collected (retro-orbital plexus). Next day i.e., on 29th the haematology and clinical chemistry studies were conducted.

5.2.1 Haematology

Ref: Tab. 5.4 for AgNP and **Tab. 5.11** for AuNP

The hematological parameters like RBC, WBC, Hct, Platelet count (Plt), Hgb, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), , Mean Corpuscular Hemoglobin Concentration (MCHC), Absolute and percentage of Reticulocytes (Retic), Mean Platelet Volume (MPV), RDW, DLC, and Blood Cell Morphology were determined using Haematology System (Bayer Health Care LLC, USA).

5.2.2 Clinical chemistry

Ref: Tab. 5.5 for AgNP and **Tab. 5.12** for AuNP

Plasma was separated in a refrigerated centrifuge at 5000 rpm for 10 min and analyzed using Roche/Hitachi 902 (Hitachi High-Technologies Corporation, Tokyo, Japan) automatic analyzer for the following parameters: Glu, D.Bil, T. Bil, Indirect Bilirubin (Ind.Bil), Creatinine (Creat), Inorganic Phosphorus (Pi), Total Plasma Protein (T.Pro), Alb, GLOB, Alb/GLOB, Gamma Glutamyl Transpeptidase (GGT), Blood Urea Nitrogen (BUN), Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline phosphatase (ALP), Total Cholesterol (T.Chol), Calcium (Ca), Triglycerides (Trig), Chloride (Cl), Sodium (Na), Potassium (K), Creatine Phosphokinase (CK).

5.2.3 Urinalysis

Ref: Tab. 5.6 for AgNP and **Tab. 5.13** for AuNP

On the treatment Day 28, urine was collected from all rats for assaying electrolytes (Chloride, Potassium and Sodium). Urine was

collected from all groups prior to sacrifice. For urine collection, the rats were placed over-night in specially fabricated cages (kept on fast) and the next morning, the collected urine was used for analysis like Leukocytes, Sp. Gr, Nitrite, pH, Glu, Proteins, Urobilinogen, Bilirubin, Ketone bodies, Appearance (color and clarity), Electrolytes (Cl, K, Na), Erythrocytes Volume. These parameters were analyzed using Combur10 test-UX strips of German made Urilux® Reflectance Photometer (Roche Dia.). Urine was also subjected to microscopic examination for sediments such as crystals, epithelial cells and casts.

5.3 Pathology studies

5.3.1 Necropsy and Tissue collection

On the day 29 all the rats were sacrificed by administering anesthesia with isoflurane, weighed, exsanguinated and subjected to detailed necropsy. On completion of the gross pathology examination the tissues and organs like Adrenal glands, Heart, Brain (cerebrum, cerebellum, medulla/pons), Kidneys, Epididymites, Liver, Pituitary, Ovaries, Spleen, Prostate, Testes, Thyroid, Thymus with parathyroids & Uterus with cervix are collected, weighed from all the group animals. The calculations for Group mean weights of organ and their ratios with respect to body wt & brain wt are done. Necropsy is done after final wt of the body are recorded and the organ wt ratios are assessed. For fixation of the different organs 10% formalin buffer is used. Percentage of organ wt ratios with respect to body wt and brain wt are recorded.

5.3.2 Histopathology

Ref: Tab. 5.7for AgNP and **Tab. 5.14** for AuNP

On the high dose group and the control animals the histopathological examinations are conducted using preserved organs and the tissues. All tissues showing gross lesions were also subjected to microscopic examination. Further, based on the microscopic changes observed in high dose animals, target organs were identified. These organs were examined microscopically in the lower dose group of animals of both the sexes.

Mayer's Haematoxylin-Eosin staining procedure is adopted for routine staining procedure after embedding the 5 micron sections with paraffin. The brains were examined microscopically at 8 levels. After fixing for minimum of 48 h 2-3 mm thick brain tissue were trimmed following the trimming guide depicted below and processed. The sciatic nerve of either side was trimmed to include both longitudinal and cross section for microscopic evaluation.

Ref: Fig. 5.1 for AgNP and **Fig. 5.2** for AuNP.

5.4 Statistical Analysis

Results of statistical analysis have been reported in the form of Mean ± SD and sample size. The statistical study of the trial data was done using the in-house developed and validated package in Excel and also using licensed copies of SYSTAT Statistical package ver.12.0. All quantitative variables like body weight (the pre-treatment body weight on the first day was considered as a covariate), net weight gain, food

consumption, laboratory investigation (haematology and clinical chemistry) and organ weight data were tested for variances in homogeneity as prescribed by Levene's test and normality as prescribed within the group before performing a One-factor ANOVA modeling by treatment groups. The data in ratios like organ weight ratios on brain and body weights were suitably transformed (logarithmic transformation) before ANOVA. Comparison of means between treatment groups and control group was done using Dunnett's 't' test when the overall treatment, 'F' test is found to be significant.

Statistically significant differences ($P \leq 0.05$), indicated by the aforementioned tests were designated by the superscripts throughout the report as stated below:

Significance ±: Lower (-)/ higher (+) is considered to be significant than control.

Experimental results and discussion

5.5 Physical and Clinical Examinations, Clinical Signs and Mortality

General clinical symptoms are studied for animals in the period of investigation. Physical and clinical examinations were carried out prior to initiation of treatment on Day 1 and at weekly intervals during the dosing. In the study we observed that there are no clinical abnormalities were found for all the concentrations used for the experiment.

5.5.1 Ophthalmological Examination

Ophthalmological examinations carried out on Day 5 of acclimatization and at the end of dosing did not reveal any eye abnormalities.

5.5.2 Cumulative Net Wt increase and Body Wt in both the sex

There were no statistically significant inter group differences in the weekly mean body weights and net weight gains in the 10, 200 and 500 µg/kg dose groups compared to the concurrent vehicle control groups in both males and females.

Food Intake

Males and Females: Food consumption in all the treated groups was comparable to the corresponding control group value throughout the trial in both the genders.

5.5.3 Clinical Pathology Investigations

i) Hematology:

Males and Females: Hematological investigation did not reveal any significant changes at any of the tested doses except for significantly lower Hb, MCH and MCHC at 200 µg/kg groups and higher platlate Values at 500 µg/kg group as compared to control group.

The above observed changes in the 200 µg/kg group was considered incidental and there is a negligible variations in the recorded factors and the similar changes were not observed at the 500 µg/kg dose group.

The hematological parameters studied is females also did not show any significant differences when the test concentration of nanoparticles is compared with the control group.

ii) Investigations in Clinical Chemistry

Biochemical investigation did not reveal any treatment-related changes in male and female rats of any group at the end of the treatment.

iii) Urinalysis

Urine sample analysis is done in all the animal groups and the variations found are negligible in all factors which are recoded in comparison to the compared to control.

5.5.4 Pathology

i) Terminal Fasting Body Weights, Organ Weights and Organ Weight Ratios

Terminal fasting wt analysis is done in all the animal groups and the variations recorded found negligible in all factors when compared to control. In adrenals of males, the significant increase in the relative weight at mid dose was considered incidental as no changes were observed at higher dose groups. Though there is a slight variation in the total wt of thymus and ovaries at the administered dose of 200 μg/ kg is considered to be unrelated to the treatment because at the higher concentration there is no variation in the values compared to control.

ii) Gross Histopathology

It is found that treatment related gross histopathological changes are nil. All the changes observed were distributed randomly in different groups. In mid dose females, the single incidence of focus in the uterus was microscopically confirmed as deciduoma. This was considered a spontaneous lesion not associated with the treatment.

iii) Histopathology

The microscopic analysis is done in both the animal sex group. From this study we can infer that there are no histopathological alterations observed in test models compared to control. The histopathological examination of high dose treated stomach, liver, kidney, pancreas and spleen from these animals were normal microscopically.

Tables of Nanotoxicology studies involved with functionalized noble metal nanoparticles.

Nanotoxicology of silver nanoparticles

Tab. 5.1 Summary of Body Weight (g) (Mean±SD)

(n=10)

Group No. Dose (µg/kg bwt /day)	Body weight (mean±SD)									
	Males					Females				
	0	1st wk	2nd wk	3rd wk	4th wk	0	1st wk	2nd wk	3rd wk	4th wk
Group-1 (Control)	235.42 ±12.41	273.11 ±15.58	298.14 ±20.22	313.52 ±22.33	335.14 ±22.46	158.84 ±7.05	169.27 ±8.66	181.84 ±7.28	188.85 ±9.39	201.14 ±10.04
Group-2 (10 µg)	239.59 ±13.84	267.62 ±13.22	291.45 ±18.64	307.77 ±22.17	328.63 ±21.86	156.85 ±5.41	164.05 ±7.93	179.8 ±6.44	188.49 ±8.8	198.48 ±6.83
Group-3 (200 µg)	236.95 ±11.48	267.48 ±11.78	293.42 ±14.24	308.77 ±14.9	329.41 ±16.73	159.12 ±7.28	170.18 ±5.96	184.59 ±7.37	194.16 ±9.90	205.46 ±8.42
Group-4 (500 µg)	237.06 ±16.99	264.54 ±19.99	287.7 ±21.79	305.96 ±23.16	325.8 ±22.12	158.88 ±9.52	171.84 ±11.38	187.27 ±13.75	196.57 ±15.53	207.71 ±16.61

Tab. 5.2 Summary of Cumulative Weekly Body Weight Gains (g)

(n=10)

Group No. Dose (µg/kg bwt /day)	**Cumulative Weekly Body Weight Gain (grams)**							
	Males				**Females**			
	1st wk	**2nd wk**	**3rd wk**	**4th wk**	**1st wk**	**2nd wk**	**3rd wk**	**4th wk**
Group-1 (Control)	35.42 ±6.28	60.44 ±12.60	75.82 ±14.97	97.44 ±15.19	10.43 ±6.02	23.00 ±4.56	30.01 ±6.40	42.30 ±6.81
Group-2 (10 µg)	32.01 ±5.26	51.86 ±9.02	68.19 ±10.63	89.04 ±10.73	7.20 ±6.10	22.95 ±4.91	31.64 ±6.13	41.63 ±4.27
Group-3 (200 µg)	30.53 ±3.52	56.46 ±6.53	71.81 ±8.96	92.45 ±10.77	11.06 ±3.28	25.47 ±3.27	35.05 ±4.32	46.34 ±3.75
Group-4 (500 µg)	31.58 ±3.44	57.45 ±6.57	72.82 ±8.66	91.45 ±9.78	11.11 ±3.30	25.55 ±3.26	34.05 ±4.36	46.88 ±3.88

Tab. 5.3 Summary of Cagewise Average Food Intake (g/rat/day)

(n=10)

Group No. Dose (µg/kg bwt /day)	Cagewise Average Food Intake (g/rat/day)							
	Males				Females			
	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk
Group-1 (Control)	23.70 ±0.87	24.28 ±1.38	24.58 ±1.32	22.06 ±1.09	14.82 ±1.08	15.70 ±0.75	16.46 ±1.49	14.36 ±1.51
Group-2 (10 µg)	22.78 ±1.09	23.28 ±1.61	23.62 ±2.07	21.38 ±1.28	13.38 ±0.84	15.34 ±0.57	16.38 ±0.35	14.40 ±0.66
Group-3 (200 µg)	23.24 ±1.36	23.80 ±0.79	24.14 ±1.18	22.36 ±1.06	13.80 ±0.89	15.64 ±0.80	17.20 ±0.79	15.56 ±0.59
Group-4 (500 µg)	22.36 ±1.56	22.66 ±2.30	23.94 ±1.26	21.88 ±1.72	14.40 ±1.93	16.04 ±1.48	17.36 ±1.25	15.52 ±1.61

Tab. 5.4 Summary of Hematological Values at Termination

(n=10)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
WBC (G/L)	7.15 ±1.41	8.76 ±1.89	8.36 ±1.93	8.90 ±1.56	6.27 ±1.49	4.92 ±0.92	5.22 ±1.19	6.01 ±1.62
RBC (T/L)	8.68 ±0.30	8.60 ±0.25	8.71 ±0.29	8.55 ±0.36	8.16 ±0.20	8.21 ±0.43	8.02 ±0.30	8.09 ±0.41
Hb (g/L)	154.90 ±4.77	156.50 ±6.20	154.70 ±3.50	153.20 ±6.65	146.60 ±2.88	148.00 ±4.57	146.20 ±5.27	144.40 ±5.58
Hct (L/L)	0.483 ±0.02	0.479 ±0.02	0.479 ±0.02	0.470 ±0.02	0.457 ±0.01	0.452 ±0.02	0.450 ±0.02	0.443 ±0.02
MCV (fL)	55.66 ±1.09	55.62 ±1.96	55.00 ±1.75	55.01 ±1.59	56.06 ±1.03	55.19 ±2.11	56.15 ±1.67	54.76 ±1.07
MCH (Pg)	17.85 ±0.46	18.21 ±0.68	17.75 ±0.44	17.94 ±0.84	17.97 ±0.58	18.06 ±0.84	18.22 ±0.51	17.86 ±0.55
MCHC (g/L)	320.70±6.22	327.40 ±7.57	323.10 ±8.08	326.10 ±10.41	320.70 ±8.31	327.20 ±9.67	324.50 ±8.77	326.10 ±7.43
Plat (G/L)	1007.4±172.1	1044.4±89.85	1110.1±113.4	1102.2±131.1	885.7 ±208.66	1014.6 ±192.9	1049.5±99.65	1051.2±141.0
PT (Sec)	15.49 ±1.73	14.35 ±0.60	14.51 ±2.06	15.70 ±1.07	15.15 ±1.95	14.70 ±0.39	15.05 ±0.95	14.71 ±1.21
APTT (S)	11.63 ±1.72	10.84 ±0.95	9.55 ±2.15	10.62 ±1.83	7.88 ±1.26	9.58 ±1.06	9.45 ±1.00	8.44 ±1.44
RDW (%)	11.46 ±0.60	11.96 ±0.67	12.00 ±0.76	11.78 ±0.59	11.40 ±0.33	11.77 ±1.05	11.27 ±0.68	11.81 ±0.41
HDW (g/L)	25.09 ±1.79	27.41 ±3.35	26.86 ±2.63	26.32 ±2.67	19.95 ±0.93	22.23 ±1.86	20.09 ±1.86	21.94 ±1.77
Neut (%)	21.41 ±6.94	18.48 ±4.67	24.10 ±4.79	21.07 ±6.55	2.63 ±0.72	3.60 ±0.89	3.21 ±0.89	3.61 ±0.77
Lymp (%)	72.59 ±7.13	76.24 ±5.03	69.13 ±5.04	73.19 ±6.83	1.62 ±1.32	1.96 ±2.00	1.12 ±0.34	1.49 ±1.01
Mono (%)	3.18 ±0.70	2.62 ±0.78	3.86 ±1.26	3.02 ±0.84	0.22 ±0.06	0.29 ±0.07	0.23 ±0.08	0.28 ±0.11
Eosi (%)	1.39 ±1.13	1.12 ±0.37	1.43 ±0.51	1.34 ±0.49	4.50 ±0.39	4.71 ±0.56	4.44 ±0.54	4.9 ±0.55
Baso (%)	0.31 ±0.09	0.32 ±0.14	0.29 ±0.14	0.24 ±0.08	2.63 ±0.72	3.60 ±0.89	3.21 ±0.89	3.61 ±0.77

Significantly lower (-) than the Vehicle Control Group
+ Significantly higher (+) than the Vehicle Control Group

Tab. 5.5 Summary of Clinical Chemistry Values at Termination

(n=10)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Glu (mmol/L)	6.91 ±0.59	6.55 ±0.78	6.42 ±0.86	6.97 ±0.72	7.14 ±0.71	6.91 ±0.95	6.44 ±0.90	7.06 ±1.08
BUN (mmol/L)	6.25 ±0.67	5.94 ±0.75	6.45 ±0.87	6.71 ±0.99	6.93 ±0.79	6.93 ±1.24	6.94 ±1.63	7.70 ±1.04
T.Pro (g/L)	65.08 ±1.20	65.09 ±2.67	65.38 ±2.36	70.33 ±2.24	66.07 ±3.56	66.86 ±2.57	66.97 ±1.86	71.92 ±2.88
AST (U/L)	80.00 ±7.53	87.60 ±37.94	79.00 ±24.86	72.00 ±8.82	72.10 ±8.33	67.30 ±7.66	66.90 ±8.21	72.40 ±7.11
ALT (U/L)	34.80 ±5.51	49.60 ±29.91	50.20 ±37.94	48.30 ±14.10	26.50 ±4.70	30.80 ±5.01	34.00 ±10.64	40.20 ±7.38
GGT (U/L)	1.30 ±0.95	1.10 ±0.99	1.20 ±1.03	1.80 ±1.23	1.00 ±0.94	2.10 ±0.74	2.80 ±1.14	2.20 ±0.63
T.Bil (µmol/L)	4.23 ±0.62	3.95 ±0.45	3.61 ±0.63	3.57 ±0.65	4.03 ±0.73	3.67 ±0.79	3.75 ±0.75	3.52 ±0.58
Creat (µmol/L)	28.60 ±3.10	27.90 ±4.68	29.50 ±4.30	30.20 ±2.66	33.10 ±3.14	33.80 ±3.26	32.00 ±4.35	36.70 ±4.50
Alb (g/L)	41.13 ±0.89	43.31 ±1.82	42.70 ±1.22	42.10 ±1.90	43.73 ±1.79	45.83 ±2.33	44.65 ±1.49	45.46 ±2.17
Pi (mmol/L)	2.37 ±0.31	2.51 ±0.30	2.37 ±0.23	2.55 ±0.26	2.16 ±0.38	2.07 ±0.29	2.16 ±0.27	1.91 ±0.17
Ca (mmol/L)	2.58 ±0.05	2.65 ±0.06	2.61 ±0.10	2.59 ±0.05	2.48 ±0.09	2.56 ±0.10	2.61 ±0.07	2.60 ±0.13
T.Chol (mmol/L)	1.35 ±0.20	1.55 ±0.31	1.41 ±0.23	1.39 ±0.11	1.42 ±0.30	1.71 ±0.30	1.84 ±0.23	2.06 ±0.33
Trig (mmol/L)	0.88 ±0.24	0.72 ±0.35	0.86 ±0.27	0.52 ±0.17	0.58 ±0.23	0.63 ±0.36	0.57 ±0.18	0.54 ±0.38
Na (mEq/L)	142.53 ±1.04	143.73 ±1.97	143.81 ±1.31	145.12 ±1.51	142.87 ±1.84	143.58 ±2.35	143.47 ±1.54	144.84 ±2.00
K (mEq/L)	3.59 ±0.23	3.56 ±0.38	3.73 ±0.22	3.78 ±0.29	3.51 ±0.35	3.26 ±0.29	3.27 ±0.30	3.30 ±0.28
Cl (mEq/L)	91.61 ±1.37	92.39 ±1.37	93.42 ±1.99	93.44 ±1.16	93.79 ±1.61	92.96 ±2.05	93.45 ±1.55	94.47 ±2.02

+: Significantly higher (+) than the Vehicle Control Group

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis

Tab. 5.6 Summary of Clinical Analysis of Urine at Termination (Incidence of parameters measured)

(n=0)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Volume (ml)	4.100	6.900	6.300	7.000	6.800	7.800	6.700	7.000
Glucose* (mmol/l)	6.000	1.000	0.000	1.000	2.000	1.000	2.000	1.000
Bilirubin*	9.000	8.000	8.000	6.000	8.000	5.000	8.000	7.000
Ketone* Bodies (mmol/l)	9.000	4.000	7.000	2.000	0.000	0.000	0.000	1.000
Specific gravity	1.031	1.020	1.022	1.018	1.017	1.015	1.016	1.018
pH	9.000	8.700	8.700	8.600	8.800	8.800	8.700	8.800
Proteins* (g/l)	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Urobilinogen* (µmol/l)	9.000	5.000	6.000	4.000	7.000	5.000	7.000	5.000
Nitrite*	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Blood*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leukocytes* (Leu/µl)	7.000	4.000	1.000	3.000	2.000	3.000	2.000	1.000

$: Mean value *: Incidences of parameters measured

Tab. 5.7 Summary of Terminal Fasting Body Weights and Organ Weights

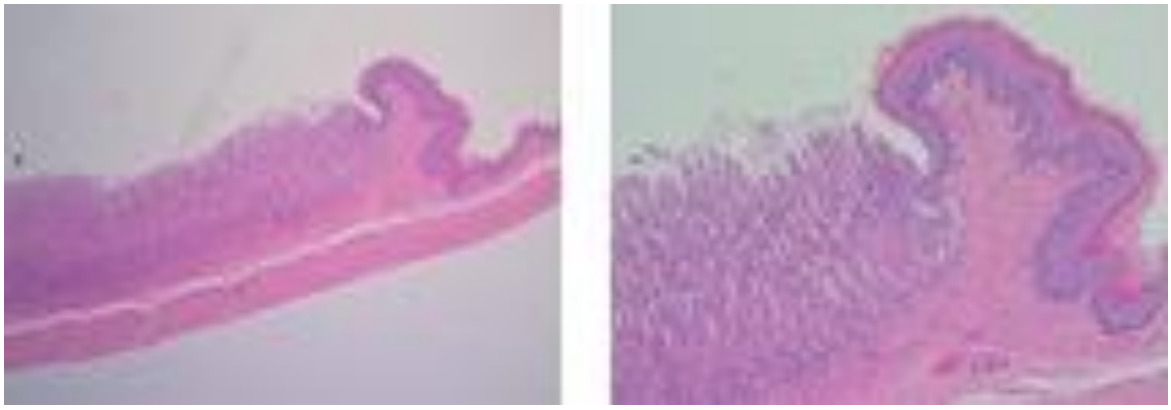
(n=10)

Group No. Dose (µg/kg Bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Fasting Bwt (g)	311.49 ±21.35	305.83 ±21.88	307.31 ±16.50	304.39 ±22.40	185.52 ±9.22	186.33 ±7.9	190.37 ±8.01	192.29 ±15.18
Adrenal glands	0.073 ±0.004	0.075 ±0.008	0.072 ±0.008	0.075 ±0.006	0.085 ±0.007	0.086 ±0.008	0.085 ±0.008	0.092 ±0.012
Testes/ Ovaries	3.371 ±0.269	3.477 ±0.172	3.298 ±0.302	3.461 ±0.310	0.102 ±0.025	0.101 ±0.014	0.103 ±0.013	0.110 ±0.020
Kidneys	2.065 ±0.201	2.188 ±0.175	2.281 ±0.186	2.103 ±0.292	1.390 ±0.102	1.416 ±0.114	1.484 ±0.073	1.435 ±0.179
Liver	8.829 ±0.964	9.397 ±1.014	9.888 ±0.866	9.894 ±0.782	5.742 ±0.267	5.854 ±0.559	6.141 ±0.721	5.013 ±0.918
Thymus	0.487 ±0.107	0.467 ±0.098	0.435 ±0.060	0.461 ±0.082	0.440 ±0.074	0.440 ±0.055	0.448 ±0.058	0.442 ±0.048
Spleen	0.681 ±0.093	0.689 ±0.080	0.623 ±0.057	0.656 ±0.083	0.457 ±0.070	0.480 ±0.103	0.483 ±0.052	0.498 ±0.087
Heart	0.954 ±0.082	0.935 ±0.085	0.922 ±0.058	0.928 ±0.079	0.672 ±0.054	0.665 ±0.047	0.692 ±0.038	0.663 ±0.052
Brain	2.015 ±0.059	2.023 ±0.078	1.981 ±0.055	1.960 ±0.046	1.886 ±0.067	1.842 ±0.034	1.835 ±0.057	1.864 ±0.050
Seminal#	1.551 ±0.191	1.713 ±0.164	1.750 ±0.283	1.695 ±0.389	---	---	---	---
Prostate/ Uterus	0.675 ±0.131	0.741 ±0.094	0.741 ±0.075	0.744 ±0.194	0.650 ±0.192	0.685 ±0.280	0.699 ±0.173	0.694 ±0.260
Epididym	1.162 ±0.073	1.179 ±0.050	1.095 ±0.100	1.135 ±0.132	---	---	---	---
Pituitary	0.009 ±0.001	0.010 ±0.002	0.011 ±0.003	0.010 ±0.001	0.012 ±0.003	0.011 ±0.001	0.011 ±0.001	0.011 ±0.002
Thyroid^	0.026 ±0.005	0.027 ±0.006	0.028 ±0.004	0.028 ±0.004	0.022 ±0.004	0.022 ±0.003	0.025 ±0.003	0.025 ±0.005

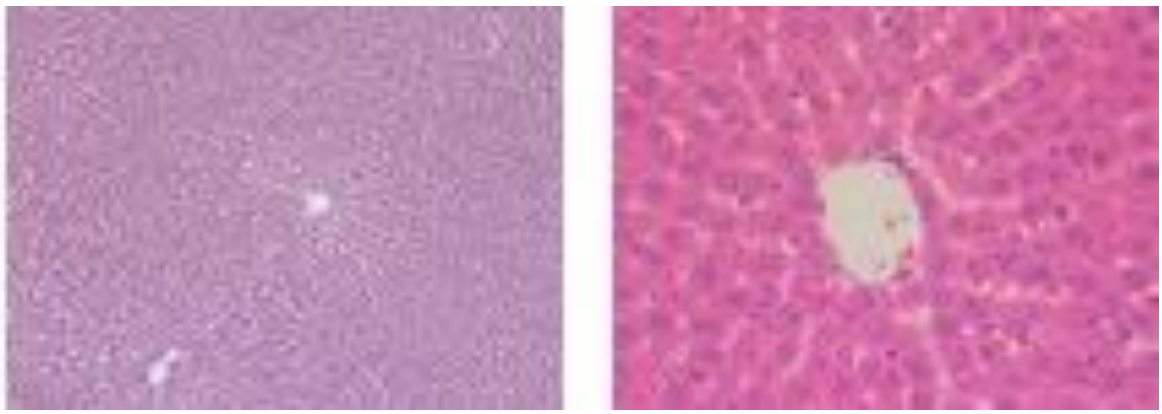
Male: #Prostate, Seminal vesicles with coagulating glands ^with parathyroid

Female: *: with cervix ^: with parathyroid

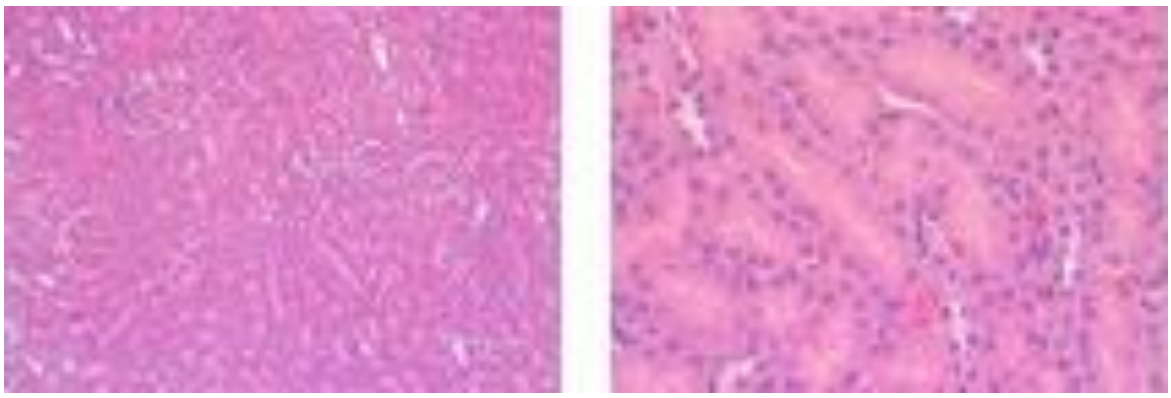
Control **500 µg/kg of AgNP**



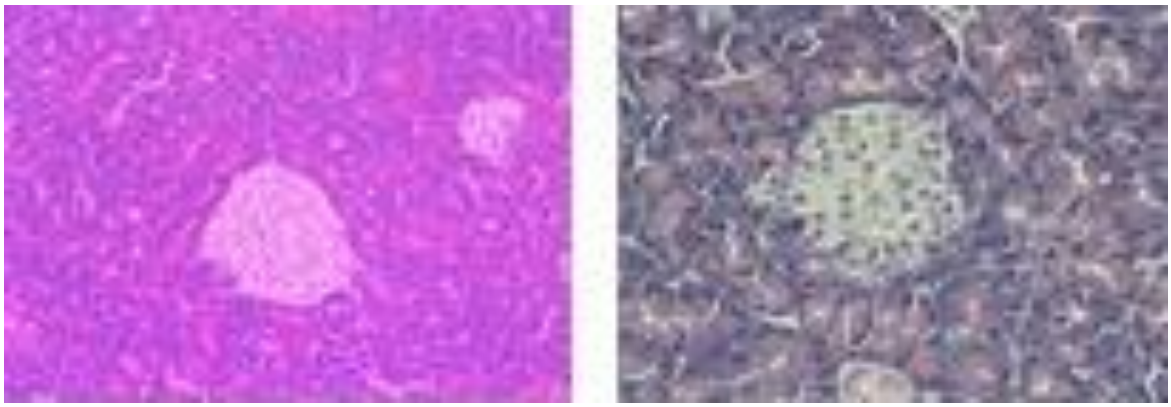
Stomach



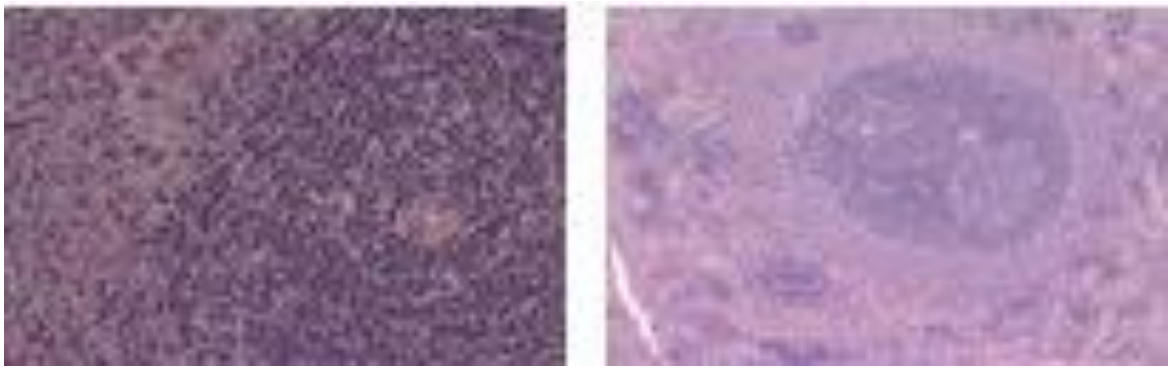
Liver



Kidney



Pancreas



Spleen

Fig. 5.1 Microscopic pictures analyzed histologically after treating with AgNP.

Tables of Nanotoxicology studies involved with functionalized AuNP

Tab. 5.8: Summary of Weekly Body Weights (g)

(n=10)

Group No. Dose (µg/kg bwt /day)	Weekly Body weight (mean±SD)									
	Males					Females				
	0	1st wk	2nd wk	3rd wk	4th wk	0	1st wk	2nd wk	3rd wk	4th wk
Gr-1 (Control)	160.77 ±5.25	172.47 ±5.21	184.32 ±8.97	191.99 ±9.27	200.96 ±10.12	220.22 ±13.73	253.23 ±17.21	284.03 ±18.68	307.11 ±22.75	324.97 ±23.16
Gr-2 (10 µg)	160.06 ±8.91	173.53 ±8.64	184.99 ±13.33	194.38 ±12.26	201.02 ±11.83	218.63 ±13.83	253.47 ±15.84	282.8 ±17.95	304.92 ±21.33	319.31 ±21.70
Gr-3 (200 µg)	163.67 ±11.98	178.05 ±13.59	190.39 ±15.70	198.24 ±15.48	206.58 ±16.78	218.76 ±14.56	256.31 ±15.55	286.90 ±19.96	308.56 ±19.92	321.87 ±26.37
Gr-4 (500 µg)	160.39 ±8.67	173.90 ±14.26	188.71 ±14.84	199.81 ±15.75	206.14 ±14.29	219.69 ±11.38	246.25 ±17.77	271.46 ±23.95	291.36 ±24.48	311.52 ±25.97

Tab. 5.9 Summary of Cumulative Weekly Body Weight Gains (g)

(n=10)

Group No. Dose (µg/kg bwt /day)	Cumulative Weekly Body Weight Gain (grams)							
	Males				Females			
	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk
Gr-1 (Control)	33.01 ±7.23	63.81 ±9.37	86.90 ±3.24	104.73 ±13.75	11.70 ±7.56	23.55 ±10.59	31.22 ±11.23	40.20 ±11.90
Gr-2 (10 µg)	34.84 ±5.65	64.17 ±7.89	86.79 ±11.80	100.68 ±9.96	13.46 ±3.96	24.93 ±6.54	34.32 ±6.73	40.96 ±6.23
Gr-3 (200 µg)	37.56 ±3.38	68.14 ±8.23	89.80 ±11.11	103.12 ±16.05	14.38 ±9.26	26.72 ±11.11	34.57 ±10.55	42.91 ±10.06
Gr-4 (500 µg)	26.57 ±9.10	61.78 ±14.82	87.20 ±15.56	91.83 ±17.10	13.51 ±9.32	28.33 ±9.50	39.42 ±12.01	45.75 ±8.31

Tab. 5.10 Summary of Cagewise Average Food Intake (g/rat/day)

(n=10, N=5)

Group No. Dose (µg/kg bwt /day)	Cagewise Average Food Intake (g/rat/day)							
	Males				Females			
	1st wk	2nd wk	3rd wk	4th wk	1st wk	2nd wk	3rd wk	4th wk
Gr-1 (Control)	23.98 ±2.60	22.96 ±0.73	23.16 ±0.86	21.96 ±0.86	15.20 ±0.93	15.44 ±0.84	16.28 ±0.80	15.44 ±0.50
Gr-2 (10 µg)	22.16 ±0.28	23.72 ±0.76	23.50 ±0.60	21.96 ±1.38	16.37 ±0.70	16.72 ±0.81	16.32 ±0.67	15.44 ±1.41
Gr-3 (200 µg)	23.74 ±0.59	23.82 ±0.86	23.50 ±0.82	21.90 ±1.53	16.23 ±0.98	15.86 ±0.54	16.52 ±1.11	16.12 ±1.28
Gr-4 (500 µg)	22.05 ±1.13	22.50 ±1.88	22.48 ±0.58	22.72 ±1.60	15.69 ±1.23	15.82 ±0.92	16.96 ±0.69	14.66 ±1.46

N is Number of cages

Tab. 5.11 Summary of Hematological Values at Termination

(n=10)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
WBC (G/L)	6.09 ±1.03	6.05 ±1.10	6.20 ±1.05	6.24 ±1.32	3.10 ±0.87	3.19 ±0.85	3.66 ±0.69	3.69 ±0.81
RBC (T/L)	9.44 ±0.60	9.24 ±0.54	9.52 ±0.47	9.10 ±0.42	8.39 ±0.34	8.50 ±0.19	8.41 ±0.31	8.51 ±0.26
Hb (g/L)	176.40 ±4.72	170.90 ±4.36	166.70 ±3.95	164.20 ±5.03	157.00 ±5.72	154.50 ±4.33	152.70 ±3.74	154.50 ±3.57
Hct (‘L/L)	0.511±0.02	0.503±0.02	0.512±0.01	0.495±0.02	0.484±0.02	0.482±0.02	0.473±0.02	0.490±0.01
MCV (fL)	54.26 ±2.50	54.52 ±1.48	53.81 ±2.28	54.38 ±1.56	57.71 ±1.83	56.70 ±1.49	56.38 ±2.16	57.55 ±1.21
MCH (‘Pg)	18.75±0.96	18.08±0.83	17.53±0.89	18.61±0.50	18.73±0.51	18.20±0.60	18.18±0.66	18.17±0.59
MCHC (g/L)	345.60 ±5.89	342.80 ±10.61	326.10 ±5.11	325.80 ±5.73	324.70 ±6.22	320.90 ±8.20	322.30 ±6.72	319.50 ±6.80
Plat (G/L)	859.9 ±77.87	923.7 ±113.66	956.7 ±71.91	1105.2±102.07	1017.1±101.09	1072.7±151.54	1041.1±102.82	1104.8±187.33
PT (Sec)	15.76 ±0.99	16.62 ±0.46	15.15 ±0.72	15.18 ±0.88	16.11 ±3.04	15.46 ±2.01	16.34 ±3.29	14.40 ±1.18
APTT (S)	16.9 ±2.21	16.3 ±3.16	17.6 ±1.82	16.8 ±1.01	13.4 ±1.63	13.1 ±1.82	12.9 ±2.72	13.28 ±2.34
RDW (%)	11.2 ±0.62	12.6 ±2.73	11.5 ±0.52	12.8 ±1.26	11.1 ±0.61	11.8 ±1.05	11.4 ±1.63	12.8 ±3.61
HDW (g/L)	22.71 ±1.29	21.02 ±1.20	22.64 ±0.93	21.09 ±1.72	20.74 ±1.53	20.90 ±0.28	22.81 ±0.82	21.53 ±1.26
Neut (%)	26.51 ±7.52	31.04 ±7.93	30.15 ±8.27	26.31 ±5.63	21.16 ±4.93	26.43 ±10.15	24.10 ±5.02	23.27 ±7.42
Lymp (%)	67.48 ±8.78	63.55 ±8.37	64.67 ±8.71	69.00 ±6.32	74.42 ±5.83	68.45 ±10.62	70.97 ±5.09	71.04 ±8.47
Mono (%)	2.61 ±0.63	2.84 ±0.70	2.61 ±1.20	1.98 ±0.76	2.03 ±0.63	2.02 ±0.37	2.07 ±0.49	2.22 ±0.84
Eosi (%)	2.64 ±1.35	1.77 ±0.53	1.83 ±0.56	1.83 ±0.81	1.63 ±0.62	2.31 ±0.95	2.09 ±0.67	2.67 ±1.69
Basop (%)	0.32 ±0.09	0.30 ±0.18	0.27 ±0.07	0.32 ±0.06	0.27 ±0.13	0.20 ±0.08	0.25 ±0.08	0.28 ±0.10

n: No. of rats Signicance +/-: lower (-) /higher compared to vehicle control group (P≤0.05)

Tab. 5.12 Summary of Clinical Chemistry Values at Termination

(n=10)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Glu (mmol/L)	06.22 ±0.21	05.93 ±0.34	06.03 ±0.49	06.04 ±0.39	05.82 ±0.40	05.93 ±0.47	05.79 ±0.37	5.96 ±0.81
BUN (mmol/L)	05.49 ±0.67	06.27 ±1.13	05.72 ±0.46	05.46 ±0.50	06.33 ±0.68	06.88 ±0.89	06.60 ±0.84	06.68 ±0.73
T.Pro (g/L)	63.69 ±2.38	64.78 ±2.89	65.90 ±2.78	62.42 ±1.81	69.64 ±2.76	70.31 ±2.69	70.33 ±3.20	68.33 ±2.07
AST (U/L)	74.80 ±5.55	76.20 ±6.58	76.90 ±4.91	72.40 ±6.45	76.70 ±11.37	76.50 ±9.42	73.40 ±4.77	68.80 ±4.92
ALT (U/L)	35.80 ±6.99	32.00 ±3.16	37.20 ±4.92	33.00 ±6.36	31.80 ±5.65	29.00 ±4.97	29.00 ±3.86	28.40 ±4.84
ALP (U/L)	101.46 ±4.30	97.29 ±2.74	98.90 ±4.84	99.47 ±5.73	85.94 ±4.84	88.21 ±2.92	86.18 ±3.51	89.03 ±2.90
GGT (U/L)	00.30 ±0.48	00.10 ±0.32	00.40 ±0.84	00.50 ±0.97	00.60 ±0.52	00.30 ±0.48	00.90 ±0.88	00.40 ±0.52
T.Bil (µ.mol/L)	02.18 ±0.43	02.45 ±0.41	02.28 ±0.51	02.25 ±0.39	02.70 ±0.47	02.70 ±0.42	03.07 ±0.40	02.53 ±0.56
Creat (µ.mol/L)	37.70 ±4.55	37.80 ±2.70	36.20 ±2.57	36.20 ±4.05	43.40 ±3.20	44.20 ±5.33	41.60 ±2.32	42.80 ±2.20
Alb (g/L)	42.44 ±1.63	42.50 ±1.50	43.96 ±1.72	40.94 ±1.59	51.81 ±2.39	50.65 ±1.46	50.41 ±3.10	49.66 ±1.95
Pi (m.mol/L)	01.47 ±0.18	01.59 ±0.21	01.55 ±0.23	01.64 ±0.22	01.19 ±0.25	01.27 ±0.26	01.36 ±0.20	01.44 ±0.23
Ca (m.mol/L)	02.96 ±0.64	02.47 ±00.24	02.17 ±0.08	02.68 ±0.74	02.09 ±0.29	02.89 ±1.01	02.83 ±0.06	02.11 ±0.71
T.Chol (m.mol/L)	01.72 ± 0.25	01.66 ± 0.25	01.81 ± 0.32	01.80 ± 0.24	01.74 ± 0.38	01.61 ± 0.28	01.79 ± 0.23	01.85 ± 0.25
Trig (m.mol/L)	0.93 ± 0.28	0.84 ±0.35	1.05 ±0.28	1.21 ±0.40	0.52 ±0.13	0.40 ±0.09	0.42 ±0.11	0.56 ±0.14
Na (m.Eq/L)	142.88 ± 1.93	143.16 ± 1.17	143.23 ± 1.23	143.01 ± 1.18	143.43 ± 1.48	142.25 ± 1.72	142.57 ± 1.70	142.98 ± 1.44
K (mEq/L)	3.87 ±0.18	3.87 ±0.32	3.83 ±0.20	3.93 ±0.24	3.55 ±0.22	3.53 ±0.18	3.62 ±0.31	3.76 ±0.33
Cl (mEq/L)	94.1 ±1.75	94.8 ±1.74	93.0 ±1.00	94.2 ±1.11	96.8 ±1.88	97.6 ±0.83	95.56 ±2.45	97.0 ±0.31

Significance: higher (+) compared to Vehicle Control Group

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis

Tab. 5.13 Summary of Clinical Analysis of Urine at Termination

(n=10)

Group No. Dose (µg/kg bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Volume (mL)	9.7	9.7	7.5	7.3	6.2	5.4	5.1	5.3
Glucose* (m.mol/L)	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Bilirubin*	2	1	3	3	6	3	5	3
Ketone* Bodies (mmol/l)	9	9	10	8	2	0	0	1
Sp. gr.	1.011	1.01	1.011	1.011	1.011	1.011	1.01	1.01
pH	8.9	8.8	8.8	8.8	8.8	9	8.8	8.8
Proteins* (g/l)	9	10	10	10	10	10	10	10
Urobilinogen* (µmol/l)	2	1	3	3	5	5	4	3
Nitrite*	10	10	10	10	9	9	10	9
Blood*	0	0	0	0	0	0	0	0
Leukocytes* (Leu/µl)	6	1	6	3	0	1	0	0

$: Mean value *: Incidences of parameters measured

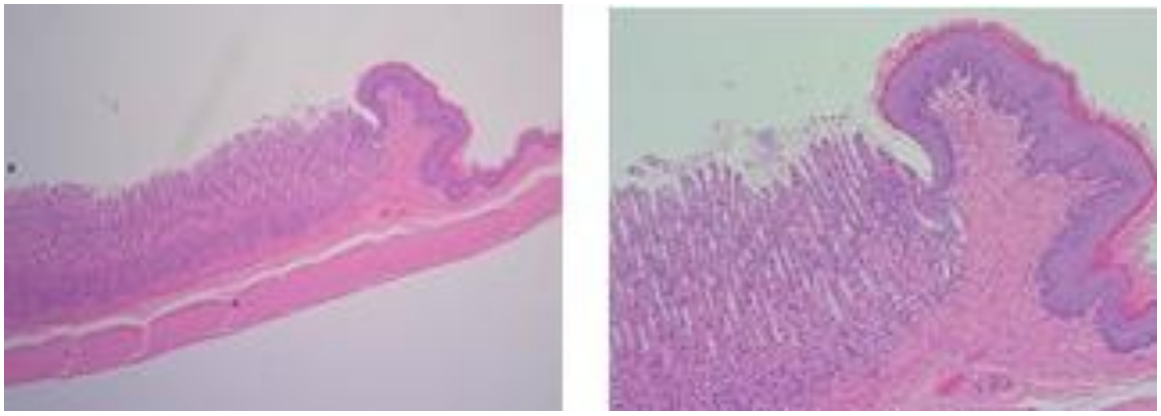
Tab. 5.14 Summary of Terminal Fasting Body Weights and Organ Weights

(n=10)

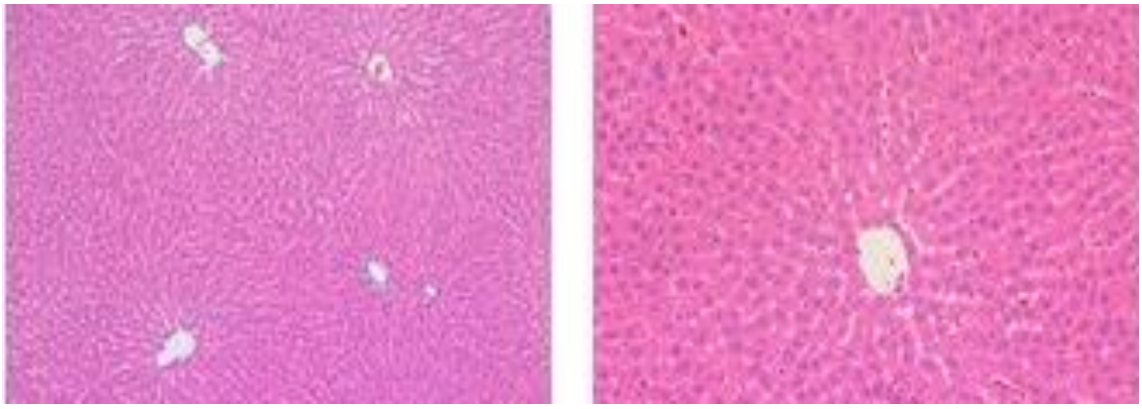
Group No. Dose (µg/kg Bwt/day)	Male				Female			
	Gr1	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Fasting Bwt (g)	231 ±9.81	230 ±10.50	221 ±12.67	237	170 ±7.61	173 ±9.54	179 ±4.83	182 ±12.19
Ad. gl.	0.042 ±0.003	0.046 ±0.004	0.045 ±0.004	0.042 ±0.005	0.054 ±0.006	0.046 ±0.008	0.051 ±0.005	0.051 ±0.006
Testes/ Ovaries	2.859 ±0.152	2.960 ±0.177	2.892 ±0.157	2.987 ±0.197	0.083 ±0.012	0.077 ±0.009	0.065 ±0.013	0.073 ±0.009
Kid.	1.713 ±0.093	1.800 ±0.112	1.613 ±0.173	1.799 ±0.161	1.306 ±0.041	1.277 ±0.064	1.301 ±0.047	1.370 ±0.069
Liver	9.602 ±1.179	9.206 ±1.100	9.005 ±0.871	10.84 ±1.427	7.053 ±0.475	7.221 ±0.523	7.303 ±0.573	7.491 ±0.729
Thy.	0.804 ±0.133	0.671 ±0.070	0.671 ±0.193	0.768 ±0.069	0.425 ±0.060	0.499 ±0.044	0.472 ±0.045	0.519 ±0.037
Spl.	0.705 ±0.082	0.773 ±0.097	0.672 ±0.092	0.734 ±0.066	0.550 ±0.056	0.586 ±0.049	0.561 ±0.033	0.630 ±0.060
Hrt.	0.914 ±0.038	0.906 ±0.039	0.838 ±0.081	0.939 ±0.053	0.722 ±0.052	0.713 ±0.044	0.756 ±0.031	0.758 ±0.062
Brn.	1.426 ±0.129	1.642 ±0.183	1.610 ±0.083	1.582 ±0.120	1.542 ±0.136	1.580 ±0.104	1.593 ±0.174	1.628 ±0.131
Seminal#	1.284 ±0.102	1.472 ±0.130	1.293 ±0.148	1.582 ±0.273	---	---	---	---
Prost./ Utr.	0.793 ±0.064	0.703 ±0.214	0.812 ±0.119	0.800 ±0.127	0.383 ±0.074	0.354 ±0.072	0.329 ±0.056	0.372 ±0.098
Epididym	0.447 ±0.048	0.453 ±0.083	0.473 ±0.039	0.441 ±0.041	---	---	---	---
Pituitary	0.010 ±0.003	0.011 ±0.006	0.011 ±0.001	0.012 ±0.009	0.009 ±0.009	0.011 ±0.001	0.010 ±0.007	0.010 ±0.005
Thyroid^	0.029 ±0.009	0.029 ±0.002	0.027 ±0.008	0.030 ±0.009	0.032 ±0.005	0.034 ±0.007	0.034 ±0.001	0.032 ±0.011

Male: #Prost. Seminal vesicles with coagulating glands ^with parathyroid
Female: *: with cervix ^: with parathyroid

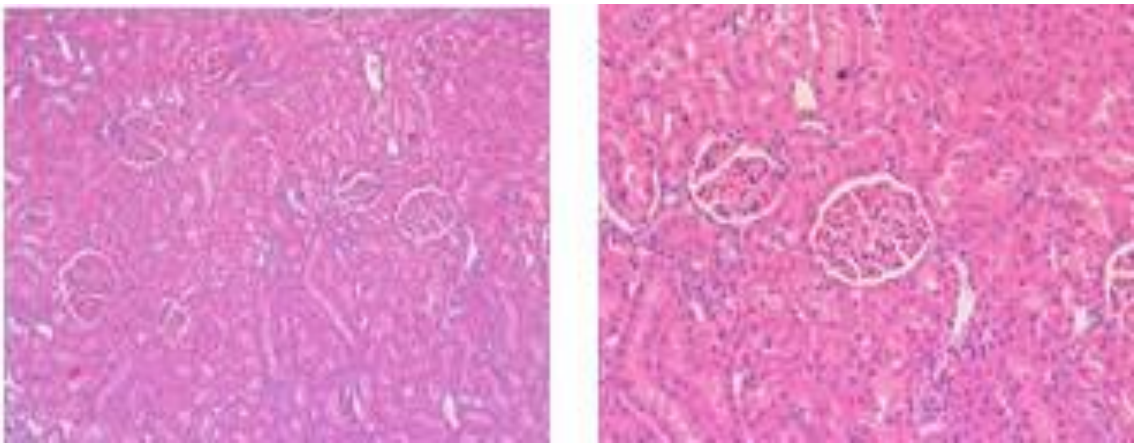
Control **500 µg/ kg of AuNP**



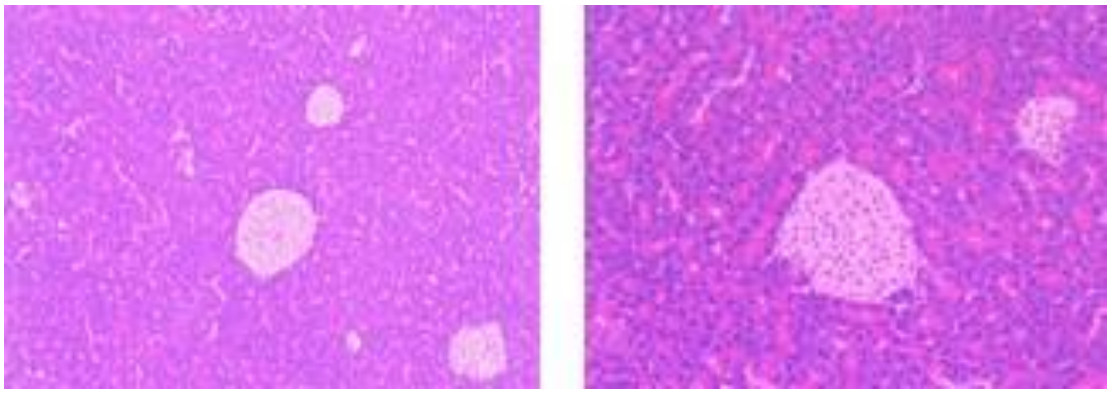
Stomach



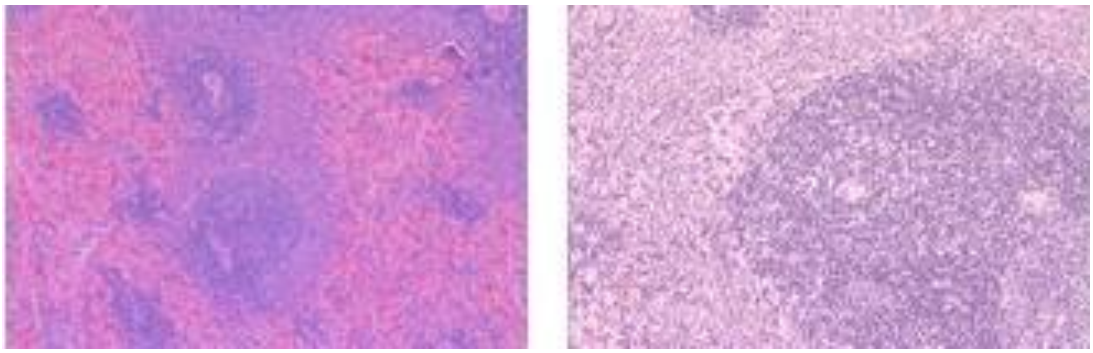
Liver



Kidney



Pancreas



Spleen

Fig. 5.2 Microscopic pictures analyzed histologically after treating with AuNP.

5.6 References

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