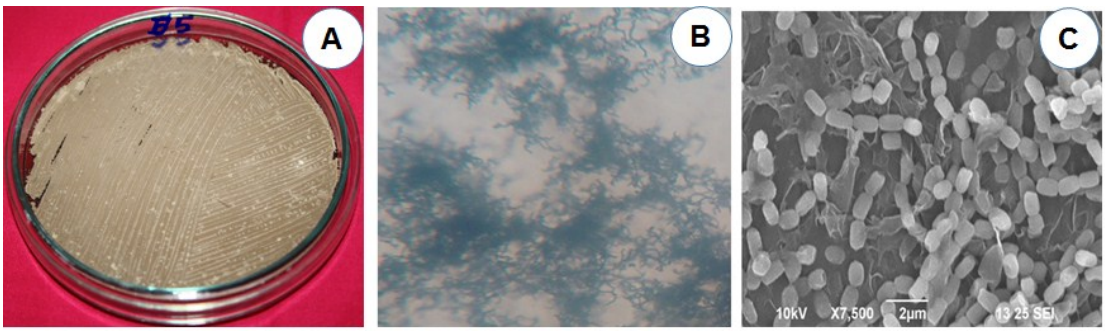
Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary materials

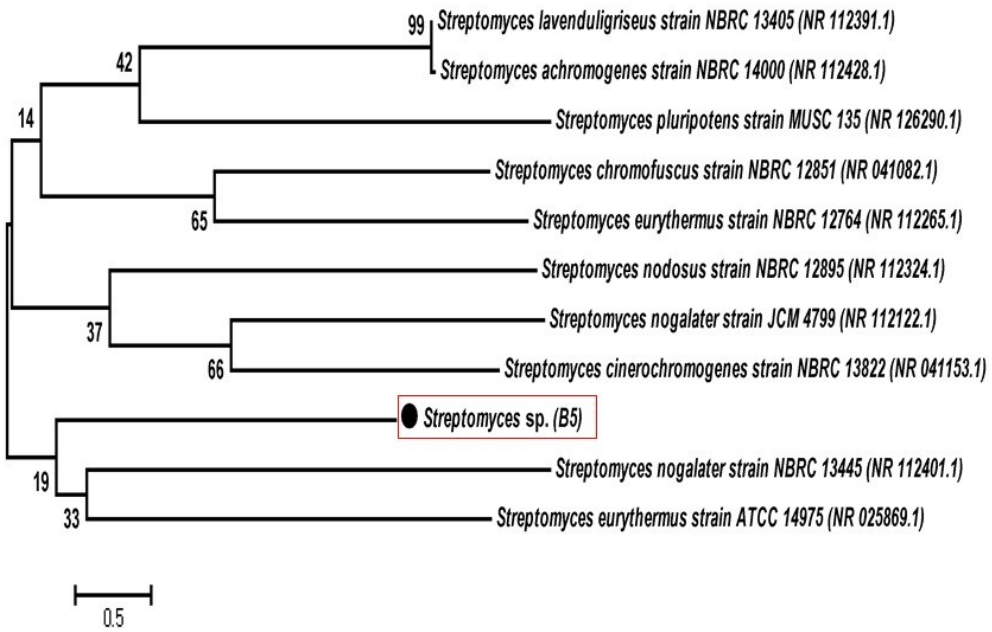
Biocompatible silver, gold and silver/gold alloy nanoparticles for enhanced cancer therapy: An *in vitro* and *in vivo* perspectives

S-Figure 1.



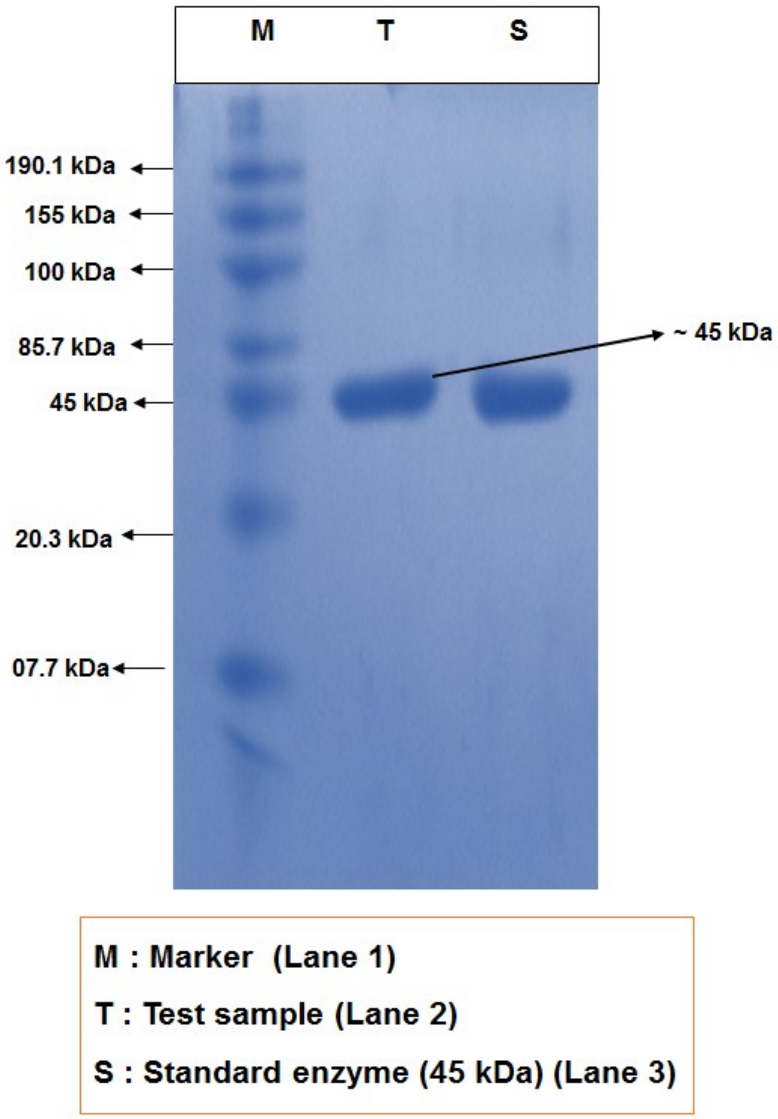
Micromorphology of *Streptomyces* strain B5 (A – ISP2 media with aerial mass, B – Light microscopic image at 400x and C – SEM image at 7,500x)

S-Figure 2.



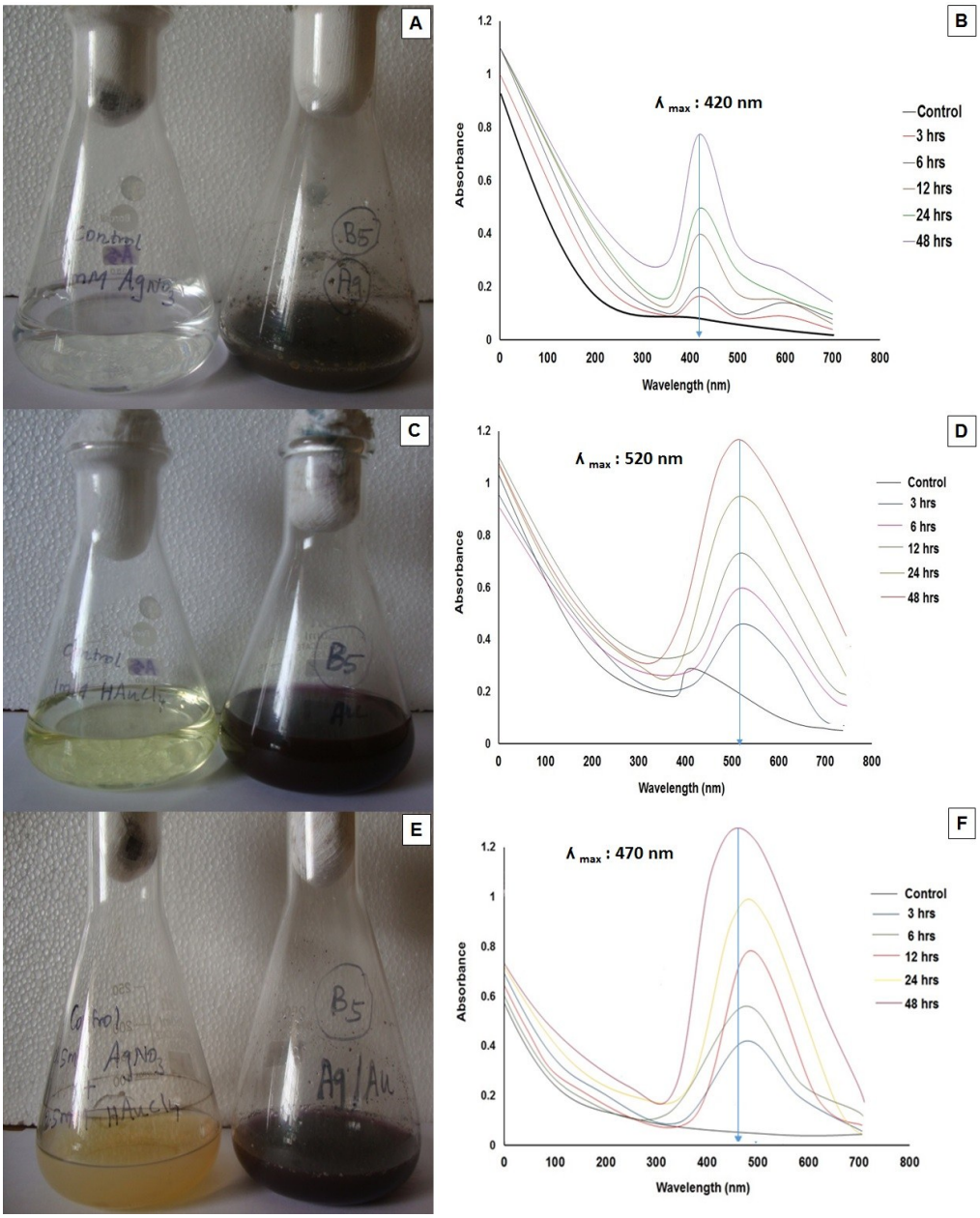
Phylogenetic analysis of *Streptomyces* sp. (B5) by Bootstrap method

S-Figure 3.



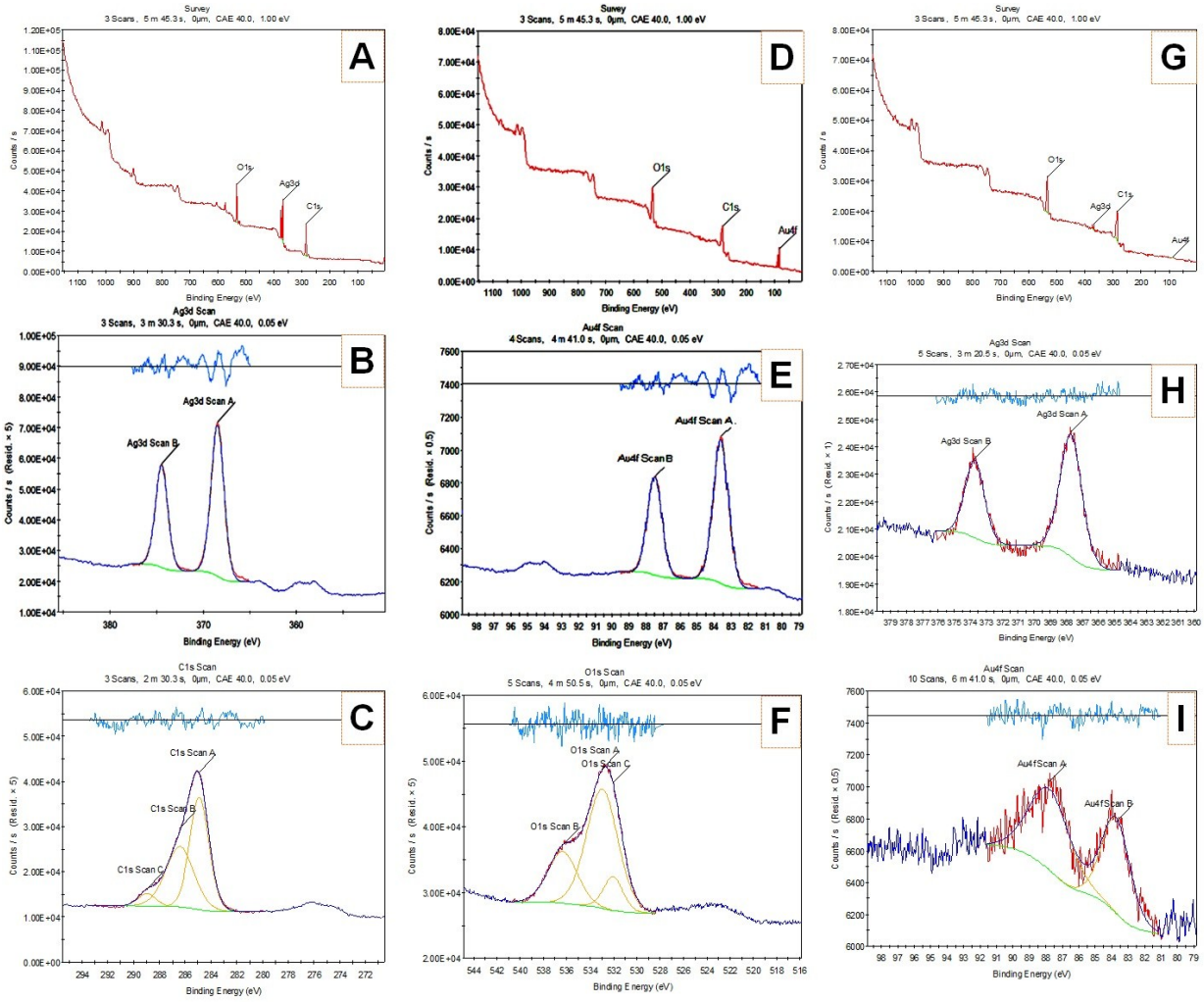
SDS-PAGE analysis of nitrate reductase enzyme

S-Figure 4.



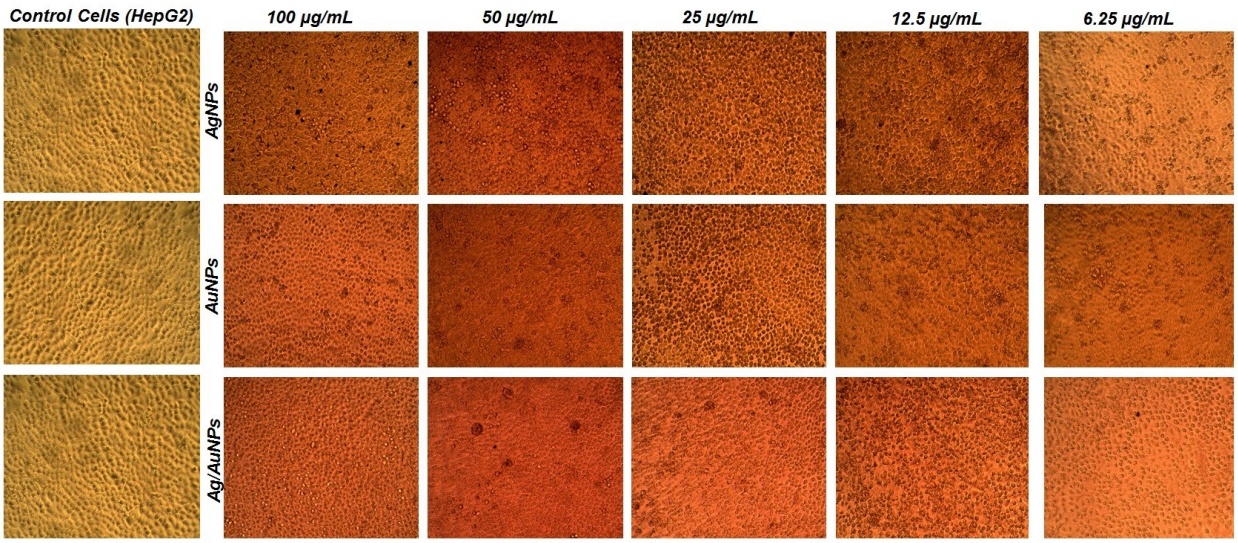
Visual observation of the reaction mixtures (A – AgNPs, C – AuNPs, E – Ag/AuNPs) and UV-visible spectral analysis of biosynthesised nanoparticles (B – AgNPs, D – AuNPs, F – Ag/AuNPs)

S-Figure 5.



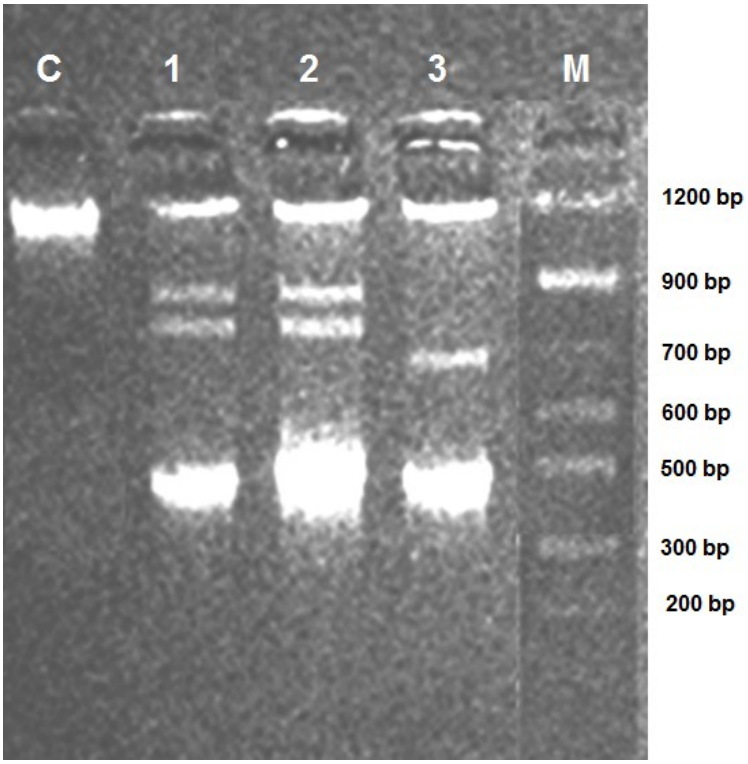
XPS analysis of biosynthesised nanoparticles, Whereas, A, D, G – corresponds to the complete spectral scan of AgNPs, AuNPs & Ag/AuNPs; B, H - corresponds to the 3d orbital scanning of Ag; E, I - corresponds to the 4f orbital scanning of Au; C - corresponds to the 1s orbital scanning of C; F - corresponds to the 1s orbital scanning of O_2.

S-Figure 6.



Anti-proliferative studies of biosynthesised nanoparticles on *HepG2* cells at different concentrations

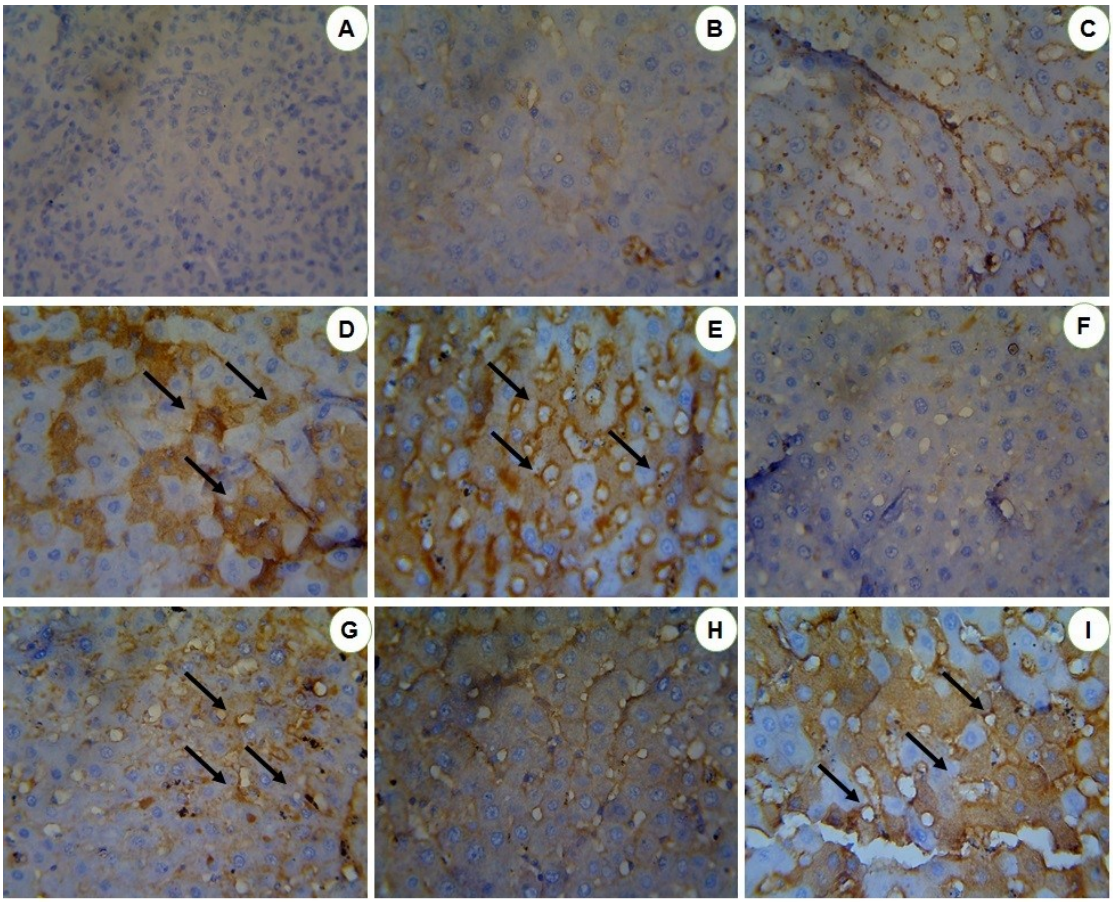
S-Figure 7.



C : Control cells, 1 : Cells treated with Ag-NPs, 2 : Cells treated with Au-NPs, 3 : Cells treated with Ag/Au-NPs, M : DNA marker

DNA fragmentation studies of nanoparticles treated *HepG2* cells

S-Figure 8.



IHC analysis of control and treatment group liver tissue samples, A : Control animals (Group I); B : Animals treated with DEN (Group II); C : Animals treated with standard drug (Group III); D : Animals treated with Ag - NPs (L.D) (Group IV); E : Animals treated with Ag - NPs (H.D) (Group V); F : Animals treated with Au - NPs (L.D) (Group VI); G : Animals treated with Au – NPs (H.D) (Group VII); H : Animals treated with Ag/Au - NPs (L.D) (Group VIII); I : Animals treated with Ag/Au - NPs (H.D) (Group IX)

S-Table 1. Cultural characteristics of potential strain B5

Characteristics	Results
Growth on different ISP media	
ISP1	Good
ISP2	Good
ISP3	Good
ISP4	Moderate
ISP5	Good
ISP6	Moderate
ISP7	Good
Carbon utilization	
Glucose	Good
Fructose	Good
Sucrose	Good
Rhamnose	Poor
Raffinose	Good
Inositol	Good
Mannitol	Good
Arabinose	Good
Xylose	Good
Cellulose	Poor
pH tolerance	
5	No growth
7	Moderate
9	Good
11	No growth
Temperature tolerance (°C)	
20	Poor
30	Good
40	Good
50	Moderate

Sodium chloride (NaCl) tolerance (%)	
1	Good
3	Good
5	Good
10	Poor

S-Table 2. Anti-proliferative activity of biosynthesised nanoparticles on *HepG2* cells

S. No	Samples	% Cell viability at different concentrations (µg/mL)					IC_{50} (µg/mL)
		100	**50**	**25**	**12.50**	**6.250**	
1	Ag-NPs	24.40	38.08	64.90	80.02	96.57	38.42
		23.92	39.34	64.21	81.78	96.32	
		24.18	38.88	64.42	80.86	96.64	
	Mean	24.167	38.767	64.51	80.8867	96.51	
	S.D.	0.2403	0.6376	0.3537	0.8803	0.1682	
2	Au-NPs	30.12	42.90	69.98	82.0	94.10	43.25
		31.09	43.04	68.82	82.18	94.36	
		30.11	42.98	68.04	81.64	93.82	
	Mean	30.44	42.9733	68.9467	81.94	94.0933	
	S.D.	0.5629	0.0702	0.9762	0.275	0.2701	
3	Ag/Au-NPs	25.0	39.99	66.62	81.10	93.32	39.20
		25.08	39.82	65.42	82.12	93.0	
		24.76	40.04	66.04	82.08	93.28	
	Mean	24.9467	39.95	66.0267	81.767	93.2	
	S.D.	0.1665	0.1153	0.6001	0.5777	0.1744	

S-Table 3. Lactate dehydrogenase and Nitric oxide assay results of biosynthesised nanoparticles on *HepG2* cells at three different concentrations

S. NO	Sample particulars	LDH release (µg/mL)	Nitrite release (µg/mL)
1	AgNPs (50 µg/mL)	0.9	0.7
2	AgNPs (100 µg/mL)	1.4	1.5
3	AgNPs (150 µg/mL)	1.5	2.4
4	AuNPs (50 µg/mL)	0.9	0.5
5	AuNPs (100 µg/mL)	1.0	1.4
6	AuNPs (150 µg/mL)	1.6	2.4
7	Ag/AuNPs (50 µg/mL)	0.9	1.1
8	Ag/AuNPs (100 µg/mL)	1.1	1.5
9	Ag/AuNPs (150 µg/mL)	3.2	2.9

S-Table 4. Physical and behavioural examinations of Ag-NPs administrated animals

Groups	Dosage (mg/kg)	Observation sign	No. of animal affected
Group - I	5	Normal	0 of 3
Group - II	50	Normal	0 of 3
Group - III	300	Normal	0 of 3
Group - IV	1000	Normal	0 of 3
Group - V	2000	Normal	0 of 3

S-Table 5. Home cage activity of Ag-NPs administrated animals

Functional and Behavioural observation	Observation	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary movement	Normal	3	3	3	3	3
Tonic involuntary movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Pedal reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

S-Table 6. Hand held observation of Ag-NPs administrated animals

Functional and Behavioural observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

S-Table 7. Mortality details of Ag-NPs administrated animals

Group	Dosage (mg/kg)	Mortality
Group - I	50	0 of 3
Group - II	50	0 of 3
Group - III	300	0 of 3
Group - IV	1000	0 of 3
Group - V	2000	0 of 3

S-Table 8. Physical and behavioral examinations of Au-NPs and Ag/Au-NPs administrated animals

Groups	Dosage (mg/kg)	Observation sign	No. of animal affected
Group - I	5	Normal	0 of 3
Group - II	50	Normal	0 of 3
Group - III	300	Normal	0 of 3
Group - IV	1000	Normal	0 of 3
Group - V	2000	Normal	0 of 3

S-Table 9. Home cage activity of Au-NPs and Ag/Au-NPs administrated animals

Functional and Behavioural observation	Observation	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)	
		Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Observation	Female mice (n=3)
Body position	Normal	3	3	3	3	Up normal	3
respiration	Normal	3	3	3	3	Up normal	3
Clonic involuntary movement	Normal	3	3	3	3	Up normal	3
Tonic involuntary movement	Normal	3	3	3	3	Up normal	3
Palpebral closure	Normal	3	3	3	3	Up normal	3
Approach response	Normal	3	3	3	3	Up normal	3
Touch response	Normal	3	3	3	3	Up normal	3
Pinna reflex	Normal	3	3	3	3	Up normal	3
Pedal reflex	Normal	3	3	3	3	Up normal	3
Tail pinch response	Normal	3	3	3	3	Up normal	3

S-Table 10. Hand held observation of Au-NPs and Ag/Au-NPs administrated animals

Functional and Behavioural observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)	
		Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Female mice (n=3)	Observation	Female mice (n=3)
Reactivity	Normal	3	3	3	3	3	Up normal	3
Handling	Normal	3	3	3	3	3	Up normal	3
Palpebral closure	Normal	3	3	3	3	3	Up normal	3
Lacrimation	Normal	3	3	3	3	3	Up normal	3
Salivation	Normal	3	3	3	3	3	Up normal	3
Piloerection	Normal	3	3	3	3	3	Up normal	3
Pupillary reflex	Normal	3	3	3	3	3	Up normal	3
Abdominal tone	Normal	3	3	3	3	3	Up normal	3
Limb tone	Normal	3	3	3	3	3	Up normal	3

S-Table 11. Mortality details of Au-NPs and Ag/Au-NPs administrated animals

Group	Dosage (mg/kg)	Mortality
Group - I	5	0 of 3
Group - II	50	0 of 3
Group - III	300	0 of 3
Group - IV	1000	0 of 3
Group - V	2000	3 of 3