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Reseach Article

Biosynthesis, Characterization, antimicrobial and hepatoprotective potential of Gold Nanoparticles of Aqueous bark extract of plant *Syzygium cumini*

Tara Shankar Basuri[1], Ranjit Mohapatra[2], N. K. Dhal[3], Sopan Kharat[4*]

[1,2]University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar – India
[3] Senior Principal Scientist IMMT, Bhubaneswar, India
[4*]Department of Botany, A.E.S. Arts, Commerce and Science College, Hingoli (MS), India

*Email: tbasuri@gmail.com, sopankharat@gmail.com

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Abstract

Drug delivery using nanomaterials have become common place in the pharmaceutical sciences and medical fields. They are now synthesised via physical, chemical, biological, and green synthesis processes. Therefore, the demand for an affordable, anodyne, and environmentally acceptable method of synthesising nanoparticles is developing. The large-scale synthesis of NPs from plant extract is far more affordable, safe, and environmentally benign. It is thought to be non-toxic and hygienic. AuNPs, or gold nanoparticles, have also been applied to cancer therapies and biosensing. This study's primary goal is to produce Au nanoparticles by using a plant extract from the plant Syzygium *cumini* (SGC), using auric chloride as reducing agent. The SGC- gold NP (SGC-GNP) was developed and optimised using Box Behnken Design. Gold Nanoparticle using SGC plant extract (SGC-GNP) synthesized using $HAuCl_4$ (X1), Plant extract ml (X2), and Reaction Time (X3) as independent parameters. The optimized nanoparticles were analyzed in UV. The synthesized SGC-GNP peaks were observed at 538 nm. FTIR spectra of SGC-Ag NPs showed intense bands at 3325, 2139, 1636, 592and 577 cm^{-1}similarly FTIR spectra of SGC-GNPs showed intense bands at 3281, 2132, 1637, 1055and 647 cm^{-1}. The particle size, Zeta potential and PDI of SGC-GNP were (38.2nm, -24.4mV and0.960mV). Gold nanoparticles were analyzed in TEM and SEM for their surface morphology. Gold nanoparticles showed an impressive Hepatoprotective and antimicrobial activity.

INTRODUCTION

In the world in 2012, liver cancer comes in at number fifty among all cancer types. In all, it is responsible for 9.1% of cancer-related deaths. In developing countries, liver cancer was diagnosed in about 83% of cases. China accounted for 50% of new liver cancer cases and fatalities worldwide. Among all cancer types, liver cancer is the fifth most common cause of diagnosis and the third most common cause of death. The death rate in emerging nations is increasing daily, with men suffering from

illness at a rate of 85% compared to women [1]. Men are almost three times as likely as women to d

evelop liver cancer [2]. During the apoptotic phase, a number of therapeutic and chemo preventive medications were used to eradicate the cancer cells. Apoptosis is a mechanism by which cells undergo death in order to control cell division and react to damage to their DNA. One of the most important steps in promoting apoptosis is the activation of caspases. As dormant zymogens, caspase is generated and is undergoing proteolytic formation that happens during death. While caspase-3 is one of the effector proteins that are crucial for initiating apoptosis and regulating the development of cancer, caspase-9 is an initiator caspase. Hepatocellular carcinomas (HCCs) account for more than 75–90% of cases of liver cancer. The incidence of liver cancer in humans is rising more quickly than any other malignancy. Risk factors for liver cancer include obesity, smoking cigarettes, excessive alcohol intake, and the hepatitis B and hepatitis C viruses [5]. The cancer patients received chemotherapy, radiation therapy, and surgery as treatments. Patients with cancer experience severe side effects from these treatments. Therefore, in order to prevent side effects, alternative medications made of natural ingredients were used in the treatment of cancer. Advances in science and technology have prompted numerous investigations into nanotechnology, with the potential to bring about significant improvements in industries including manufacturing, health care, and medicine. In biomedical applications, bio-reduced gold and silver nanoparticles have become the most significant of all the nanoparticles. The shape and size of a material's surface are crucial in determining its chemical, physical, and electrical characteristics [1]. The two most popular and reliable nanomaterials for biology-related biomedical research and applications are Ag and Au nanoparticles [2]. A gold nanoparticle's characteristics differ from its mass because the former is a wine-red solution while the latter is a yellow solid. It is possible to produce gold nanoparticles in a range of forms. The characteristics and properties of gold nanoparticles are greatly influenced by their size and form. Gold nanoparticles have transformed the field of targeted medicine because of their wide range of applications in detection, targeted drug delivery systems, and therapies due to their very tiny size,

physical and chemical characteristics, high surface area, stability, and non-cytotoxicity optically [3, 4]. Because gold nanoparticles may be manufactured in a variety of sizes and forms, their preparation is not only comfortable but also very flexible [2]. Researchers from France developed a method to create luminous gold nanoparticles inside of human hair. They demonstrated that even after several washings, the gold color persisted [5]. A significant portion of the research is focused on the synthesis, stability, and functionalization of GNPs. Various oligonucleotides, peptides, antibodies, and lipids are standard species utilized for functionalization; the most frequently reported reduction agents are sodium citrate, transferrin, and natrium borohydride. The main reasons for the interest in GNPs are their excellent biocompatibility, good control over their sizes, shapes, and optical properties, and relative ease of synthesis [6].

Methodology

Preparation of extracts of plant materials

The different extracts of the drug will be prepared by different extraction techniques such as Soxhlet extraction, hot maceration etc. The extracts will be separated from the marc by filtration, concentrated to dryness and stored at -20°C until further use.

Synthesis of gold/plant extracts nanoparticles [8]

20ml of plant extract will be added to 10 ml of triple distilled water in a conical flask. Chloro auric acid solution 1M will be added to the mixture with vigorous stirring in a magnetic stirrer at normal room temperature. After 24 hours, colour of the final mixture started to become slightly brown which indicated the formation of GNPs.

Characterisation of plant extract mediated gold & silver nanoparticles [8]

UV-Vis Spectroscopy

Ultraviolet-visible spectroscopy or ultraviolet-Visible spectrophotometer (UV-Vis) will be used in the present study to find out the absorption of nanoparticles in the UV-Visible spectral region. This means it uses light in the visible and adjacent near-UV and near-infrared (NIR)) ranges. The absorption in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

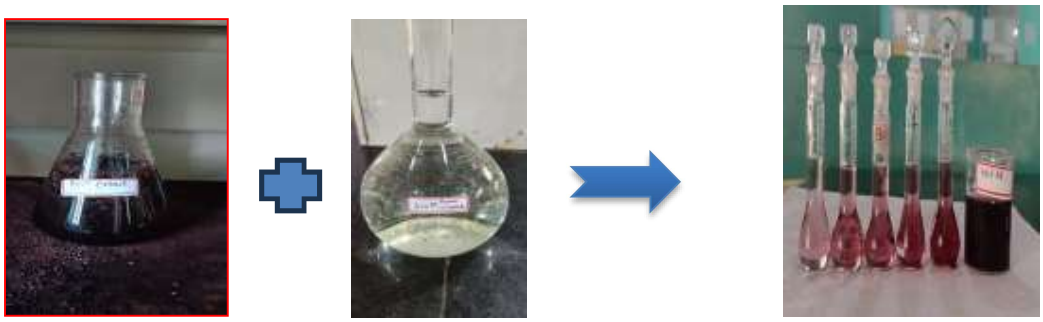


Figure. 1. (a) Plant extract (b) 1mM Auric chloride (c) Gold nanoparticle formation

Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscope (SEM) analysis will done using SEM machine. Thin films of the sample will be prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution will be removed using a blotting paper and then the film on the SEM grid will be allowed to dry by putting it under a mercury lamp for 5 min.

Fourier Transmission Infra-Red Spectroscopy (FTIR)

FTIR is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It will be used to analyze the bio molecule and also bonding interaction between molecules of the gold nanoparticles. IR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. When an infrared light interacts with matter, chemical bonds will show stretch, contract and bend form. These chemical functional group tends to adsorb infrared radiation in a specific wave number range of the structure of the rest of the molecule.

X-Ray Diffraction (XRD) Measurements

The phase formation of bio reduced silver nanoparticles will be studied with the help of XRD. The diffraction data of thoroughly dried thin films of nanoparticles on glass slides will be recorded on X-ray diffractometer.

Acute Toxicity Studies [5]

The acute toxicity studies will be carried out as per stair case method. Fifty male rats will be divided into five groups of 10 each and will be administered with aliquot doses of the extracts orally (100, 150, 200, 250 and 300 mg/kg). Mortality will be noticed. The LD50 of the extracts will be found out. One-tenth of this dose will be selected as the therapeutic dose for the evaluation

Evaluation of Hepatoprotective activity [4]

Screening method:

Five groups of Wistar rats animals containing six each will be used for the study. The animals from Group I served as the control and received the vehicle 1% w/v gum tragacanth at a dose of 1 ml/kg/day of p.o. for 14 days. Groups II–V received 0.1 ml/kg/ day i.p. of CCl4 for 10 days. Group II received CCl_4 treatment only. The standard drug Silymarin will be administered to Group III animals in the dose of 100 mg/kg/ day p.o. for 14 days. While, Groups IV and V will be treated with extracts, p.o. (as per acute toxicity studies) for 14 days, respectively. The CCl_4,

silymarin and the extracts will be administered concomitantly to the respective groups of animals.

Grouping & Treatment Protocol:

Group – I: Control group: -Animals received 1% w/v gum tragacanth at a dose of 1 ml/kg/day of p.o. for 14 days.

Group- II: Rats will be treated with 0.1 ml/kg/ day i.p. of CCl4 for 10 days

Group – III: Rats will be treated with 0.1 ml/kg/ day i.p. of CCl4 for 10 days & standard drug in the dose of Silymarin 100 mg/kg/ day p.o. for 14 days.

Group – IV: Test group IV: Rats will be treated with extracts.

Group – V: Test group V: Rats will be treated with extracts.

Assessment of hepatoprotective activity

All the animals will be killed on day 14 under light ether anaesthesia. The blood samples will be collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum will be separated at 2500 rpm for 10 min and biochemical investigations will be carried out to assess liver function viz., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase. The results are expressed as mean±SEM of six animals from each group. The data will be evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. P values <0.01 were considered statistically significant.

Histopathology

After draining the blood, liver samples will be excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections will be taken at 5 mm thickness, processed in alcohol–xylene series and will be stained with alum hematoxylin and eosin. The sections will be examined microscopically for histopathological changes.

Results and Discussion

Characterization of *Syzygium cumini* Extract Gold Nanoparticle SGC-GNPs

UV-Visible spectroscopy

UV-Vis spectroscopy is an important technique to observe the formation of GNPs by monitoring the optical properties and electronic structure of the synthesized nanoparticles. The electron cloud of a nanoparticle can oscillate on the surface of the nanoparticle, which absorbs electromagnetic waves at a particular frequency. This phenomenon is called surface plasmon resonance (SPR) and recorded as electromagnetic wavelengths by UV-Vis spectrophotometer (Fig. 2) shows the optimization of incubation time during GNPs synthesis. Based on the spectra, UV wavelengths of SGC-GNP-B (GNPs from Bark extract) were taken from 15 min until 24 h of incubation. The peaks intensified as the incubation time increases. The increase in intensity of the wavelength absorbance is due to the increasing number of nanoparticles formed from the reduction of silver ions and biomolecules in the aqueous plant extract solution. The analysis for extract concentration was performed at 25°C for 24 h with extract concentrations at 2%, 4%, 6%,8% &10% (v/v). The synthesized SGC-AgNP-B peaks were observed between 538 nm, the sharpness of the peak indicates the formation of spherical shape of synthesized GNPs and homogeneous distribution.

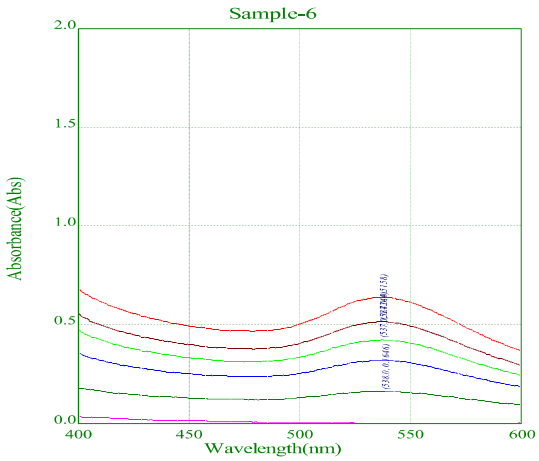


Fig.2. UV -Visible spectra of gold nanoparticle of *S. cumini*

FT-IR Spectroscopy SGC-GNPs

The interaction of SGC on the surface of the SGC-GNPs was confirmed by FT-IR spectral analysis. The IR spectrum of SGC showed intense bands at 3324, 2132, 1636, 633 and 590 cm−1 (Fig. 3) and that of SGC-GNPs showed intense bands at 3281, 2132, 1637, 1055and 647 cm-1 (Fig.4). The significant difference observed between the of FT-IR bands of SGC and SGC -GNPs was due to the reduction process. The strong and intense band centered at 3324 cm−1 was assigned to stretching vibration mode of –OH groups in SGC (Fig. 4) and another band at 2132 cm−1 was assigned to the stretching vibration of C-H groups.

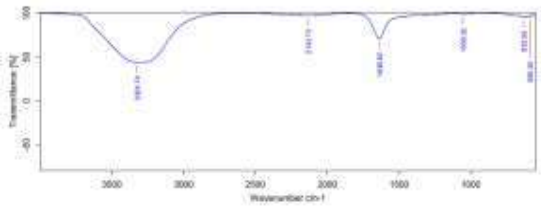


Fig.3. FT-IR Spectroscopy SGC Extract

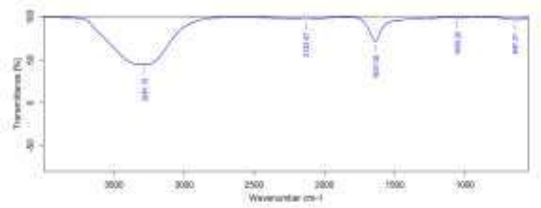


Fig.4. FT-IR Spectroscopy SGC-GNP

XRD studies SGC-GNPs

The XRD pattern of the **SGC**-GNPs is shown in the Fig. 5 and various Bragg reflections clearly indicated the presence of (12.5), (23.4) and (25) sets of lattice planes that can be indexed to a face-centered- cubic (fcc) structure for silver. Hence, from the XRD pattern, it is clear that **SGC**-GNPs formed were essentially crystalline in nature. In addition to the Bragg peaks representative of fcc GNPs, additional unassigned peaks observed suggested the crystallization of bioorganic phase on the surface of SGC –GNP.

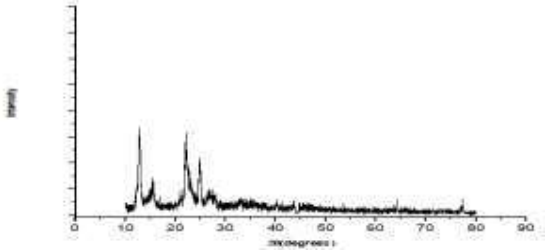


Fig.5. XRD studies SGC-GNPs

DLS (Particle size) and zeta potential of SGC-GNPs

The data of Dynamic light scattering studies indicated uniformity in the distribution of particles and the average size of the DLS-GNPs (Fig.6) to be 38.2 nm. The PDI is 0.960. The stability of the SGC-GNPs was inferred from zeta potentiometer measurements. A zeta potential value of -24.4 mV **(Fig. xxx)** essentially indicated avoiding of aggregation of nanoparticles in suspension.

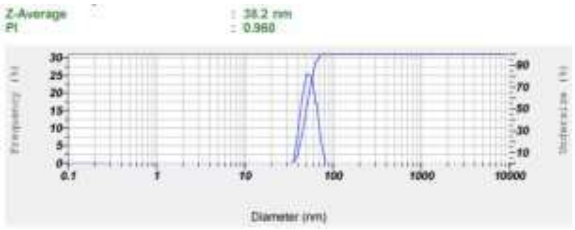


Fig.6. Particle size of SGC-GNP

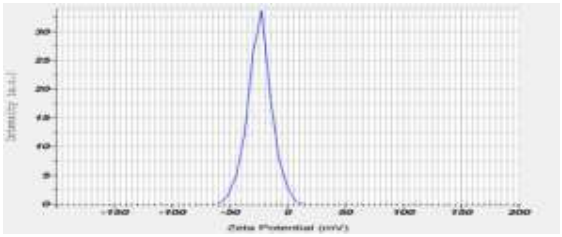


Fig.7. Zeta Potential of SGC-GNP

SEM Analysis SGC-GNPs

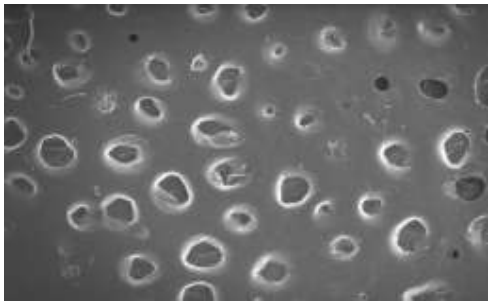


Fig.8. SEM analysis SGC-GNPs

The TEM images of SGC -GNPs are shown in Fig. 9. From the 100 nm magnification of nanoparticles the morphology of SGC-GNPs was found to be spherical in shape. The histogram of the size distribution of SGC-GNPs displayed broad particle size distribution and the size of nanoparticles as 38.2 nm (Fig. 9).

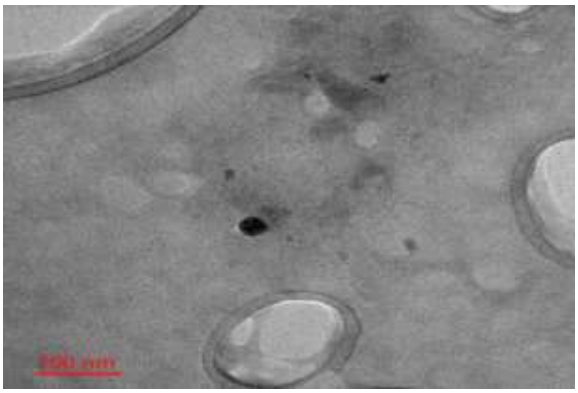


Fig.9. TEM Image of SGC-GNPs

HEPATOPROTECTIVE ACTIVITY OF SGC-GNPs

Acute Oral Toxicity Study in Rats

1. Study Title

Acute Oral Toxicity Study of Gold Np.

2. Guideline

Acute Toxic Class Method (OECD-423, 2008).

3. Testing Facility

Systemic Life Sciences, Hyderabad.

4. Summary

This study was designed to evaluate acute oral toxicity potential of Gold np in Sprague Dawley (SD) rats. Considering the low acute toxicity potential of tested drug, a limit test at the dose of 2000 mg/kg bw (i.e., maximum feasible dose) as per OECD-423 was conducted. Animals were administered with single dose of formulation in step 1 and observed for lethality and toxic signs & symptoms at 30 minutes,1 hour,2-hour,4 hour and 48 hours. As no lethality and mortality was observed following treatment with Gold np in 3 consecutive animals in step 1 respectively, step 2 viz confirmatory test was carried out in another three animals with same dose level 2000mg/kg. All the Six animals were sacrificed on Day 15 and necropsy was performed. No treatment related gross pathological abnormality was observed. Under the given conditions, no toxic signs and symptoms or mortality was observed at the dose of 2000 mg/kg bw. Therefore, oral LD50 of Gold np in female SD rat was estimated to be greater than 2000 mg/kg body weight.

5. Test Substance Details

Product name:

Gold Np

Manufacturer and Supplier:

Anurag

Physical State:

Liquid

6. Experimental Design

Name of species:

Albino rats

Strain of the animals:

Sprague Dawley rats

No. of animals used:

Maximum 12 female rats as per the limit test of OECD-423.

Weight range:

120±10g

Route of administration:

Oral

Date of initiation:

20.02.2024

7. Animal Husbandry

Female rats were randomly selected and housed individually in polycarbonate cages with stainless steel wire mesh tops and having sterilized corn cob bedding. The room temperature was maintained at 22 ± 3° C with 30 - 70 % relative humidity. Animals were kept at 12 hours light & dark cycle. Standard pellet feed and water were provided *ad libitum* to the experimental animals except stated otherwise.

8. Acclimatization

Rats were acclimatized for 5 days to the laboratory conditions before the commencement of the study.

9. Experimental Procedure

Rats were fasted overnight prior to dosing. Three female rat was orally administered with 2000

mg/kg bw of Gold np via using stainless steel feeding cannula. The rat was survived; therefore, three additional rats were administered with 2000 mg/kg bw, 48-h after the dosing of first three animal. Since both Step 1and step 2 rats survived, after administering with 2000 mg/kg bw 48-h after the dosing of previous step 1 animals. Therefore, all the Six animals were carried to full 14-day observation without dosing to further animals. All the Six rats were sacrificed after 14-day observation period and were subjected to necropsy. All external and internal lesions (if any) were carefully observed and recorded. As, no toxic lesions occurred, no tissue samples were taken for histopathological examination.

10. Observations

All experimental animals were observed for 14 days. Observations were made at least once during first 30 minutes with special attention during first 4 hours on the day of dosing and once daily thereafter for the total of 14 days. Body weights and feed consumption were recorded prior to dosing and thereafter at different time interval. Necropsy was performed and organs were collected and organ weigh data was recorded.

11. Results

Individual clinical observations and mortality data are given in Table 1.

Mortality:

There were no deaths either on the day of treatment or throughout the 14-day post-treatment observation period.

Clinical observations:

No signs of systemic toxicity were observed throughout the observation period.

Body weight:

Individual body weights are given in Table 2. None of the animals lost body weight and all rats showed expected gains in body weight over the study period.

Feed consumption:

Feed intake data is shown in Table 3.

Haematology:

Haematology data is shown in Table 4.

Biochemistry:

Biochemistry data is shown in Table 5.

Necropsy:

All the Six animals survived until the scheduled necropsy on Day 15. No abnormalities were noted and all organs of rats proved to be free of Gold np treatment related gross pathological changes. Individual necropsy findings are given in Table 6.

12. Conclusions

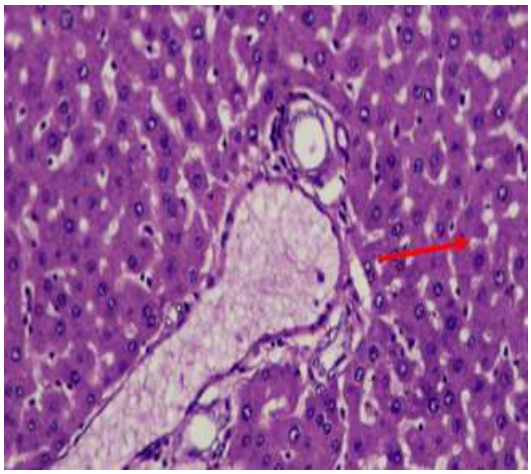
No overt toxic sign and symptoms or mortality was observed in any of the animals at the dose of 2000 mg/kg bw. Based on the study data, the oral LD50 of the Gold np in the female SD rats was estimated to be greater than 2000 mg/kg body weight.

Statistical analysis

Data analyzed with One way ANOVA followed by Tukey's multiple comparison tests showed significant influence of Gold Extract on serum liver parameters AST, ALT, ALP (U/L), Total Bilurubin (mg/dL), and Total protein (g/dL). All the parameters AST, ALT, ALP, Total Bilurubin significantly elevated and Total protein significantly decreased in toxic control group (####$P<0.001$) compared to normal control group

which depicts the excellent model establishment with CCl4 in this study. Silymarin and Gold extract treated rats significantly shows decreased levels of AST, AST, ALT, ALP (U/L), Total Bilurubin (mg/dL), and elevated Total protein (g/dL) content (Table 1). While Silver extract treated rats showed only significant influence on AST, ALT and T. Bilurubin and further couldn't improve ALP and total protein significantly.

Histopathology



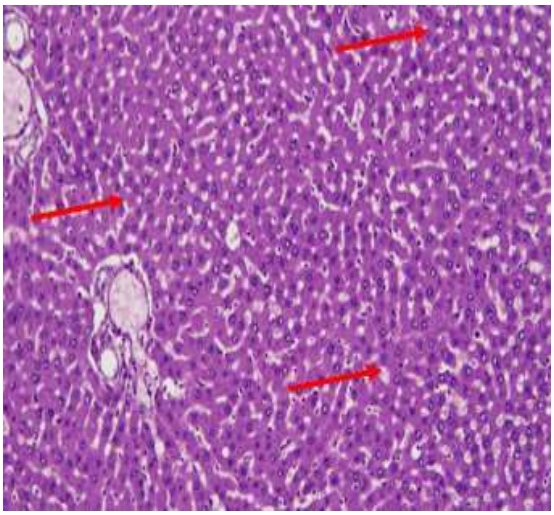
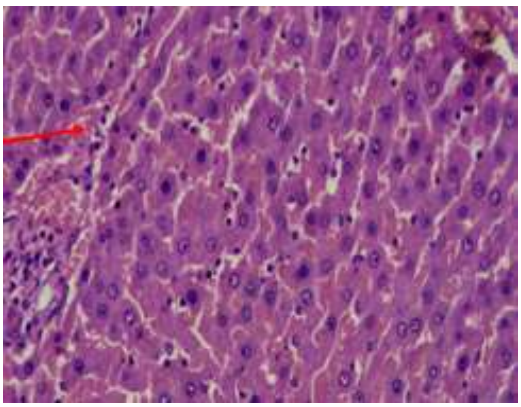


Fig. Normal morphology of hepatocytes were observed in the portal, periportal and centri lobular region of liver – arrow. No abnormalities were observed in liver.



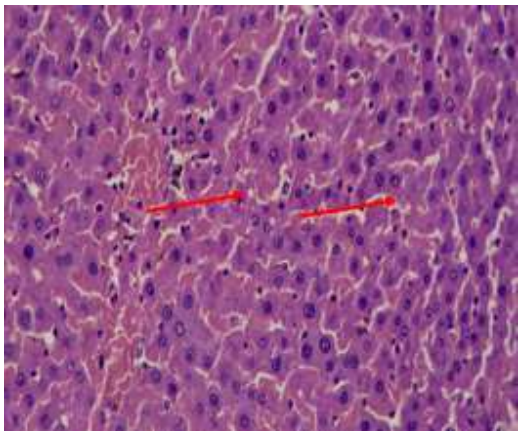
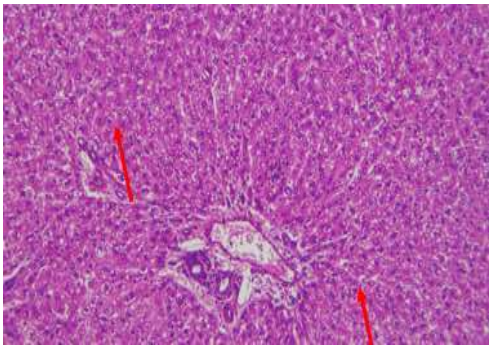


Fig. Mild sinusoidal haemorrhages was observed in sinusoidal spaces of liver



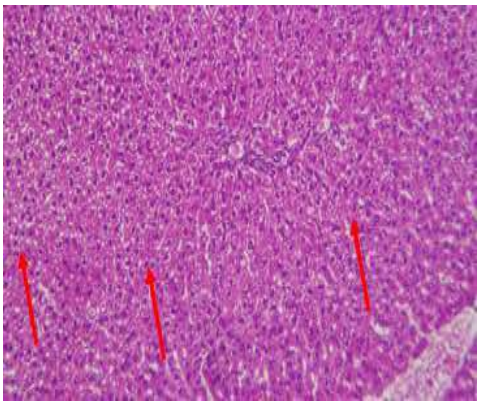


Fig. Hepatocytes are appeared normal in portal, peri portal and centri lobular region of liver – arrow (Treatment with Gold NP Extract).

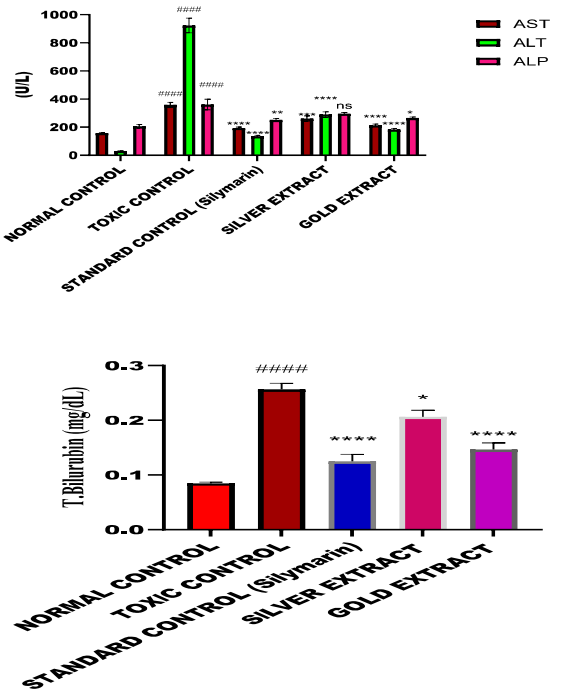


Fig. Graph showing Activity %

Conclusion

The present work reports a simple, novel and successful synthesis of silver and gold nanoparticles using *Syzium cumini* aqueous bark extracts as a novel reducing and stabilizing agent of gold salts. UV-Vis spectral analysis confirmed the surface plasmon resonance of biosynthesized Au-NPs. The DLS and TEM images studies had shown that the synthesized Ag-NPs and Au-NPs have a size below 38.2 nm respectively. Zeta potential value for biosynthesized Au-NPs was (-24.4) indicating the stability of the nanoparticles. From FT-IR spectra, it was found that biomolecules responsible for capping and stabilization of Au-NPs were heterocyclic water-soluble compounds present in the *Syzium cumini* aqueous extracts. In this study CCL4 induced hepatotoxicity in rats; Gold extract has an excellent hepatoprotective activity, almost identical with standard drug silymarin. while Silver extract has a moderate hepatoprotective activity.

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