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DEVELOPMENT AND CHARACTERIZATION OF SILVER NANOPARTICLE OF stevia rebaudiana BERT EXTRACT FOR HEPATOPROTECTIVE ACTIVITY IN PARACETAMOL INDUCE TOXICITY

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ABSTRACT

Stevia rebaudiana Bert is a medicinal plant that is widely used in traditional medicine owing to its broad range of therapeutic activities. Despite its promising pharmacological activities, using plant extracts has several limitations that can be overcome using pharmaceutical nanotechnology. The aim of this study was to systematically investigate the effect of silver nanoparticles (Ag-NPs) on the hepatoprotective activities of *stevia rebaudiana* B. extract. Silver nanoparticles (Ag-NPs) were prepared using the green synthesis method and characterized in terms of size, polydispersity index, zeta potential, encapsulation efficiency, high-performance thin layer chromatography (HPTLC), X-ray diffraction, differential scanning calorimetry, and release profile. Transmission electron microscopy was used to observe the morphology of the nanoparticles. The mean particle size, zeta potential, and encapsulation efficiency of optimized NA-CSNPs were 137.5 nm, 0.226, -33.76 mV, and 50.12%, respectively. The release profile of the Ag-NPs was mainly dependent on the pH of the surrounding medium, and the *Stevia rebaudiana* B. extract was released in a controlled manner over time. However, hepatic stellate cells, hepatocytes, and It cells are directly and indirectly affected during hepatotoxicity. Their over-activation revealed another approach to liver injury due to the influence of any toxicant. Taken together, the results of this study highlighted the advantages of using NPS as a nanocarrier for herbal extracts, thus providing a potential strategy for improving plant-based therapeutics.

Key-words: Natural extract**,** silver nanoparticles, Anti hepatoprotective activity, *Stevia rabaudiana*

1. Introduction

The metabolism and detoxification of substances that enter the body and induce hepatic damage, which can result in life-threatening disorders, are critical liver functions. As a result, the effects on the liver have been the focus of the main toxicological issues linked to a number of disorders (Ramappa V, Aithal GP, et al., 2013). Hepatotoxic substances often damage liver cells by causing oxidative damage (Singh D, Cho WC et., al 2015). Medications that can be used to treat liver problems are synthetic and natural in types. Liver disorders have traditionally been treated using natural therapies. As a result, the recent importance of plant-based herbal medicines' preventive benefits against drug-induced toxicity has skyrocketed (Xiong F, Guan Y-S, et al., 2017).

Stevia, an amazing plant from the Amazonian rainforest, is the sweetest gift from nature and belongs to the Asteraceae family. Honey yerba, honey leaf, sweet herb, and sweet leaf are further names for stevia. Although it is also commonly farmed in Central America, Israel, Australia, Japan, and China, stevia is a South American plant that is native to Paraguay and Brazil (Sharma N, Kaushal N, et al., 2006). Stevia is unique in that its leaves contain the sweet Diterpene glycosides rebaudioside A, rebaudioside C, stevioside, and glucoside. Rebaudioside A is 250–450 times sweeter than sucrose, and stevioside, a white crystalline molecule derived from Stevia, is 150–300 times sweeter (Vinatoru et al., 2001)

Traditional methods such as maceration, infusion, or decoction have been used to extract the glycosides from Stevia rebaudiana leaves. These methods either required high processing times and limited efficiency (maceration), or they were subject to thermal destruction (infusion and decoction). It is employed in the treatment of a number of ailments, including cancer) diabetes obesity, cavities, hypertension, fatigue, and depression, as well as in preparations for the cosmetic and dentistry industries (Duke JA, Chan P, Tomlinson B, et al., 2000).

It has hypertensive vasodilator, sweetening, taste-improving, antifungal, antiviral, anti-inflammatory, antibacterial, and urination-increasing bodily functions. The findings of numerous toxicological investigations into the potential mutagenic and genotoxic effects of Stevia extracts on bacterial cells and other mammalian species were recently reviewed (Smirnova MG et al., 2001, Huxtable RJ et.al 2002). These studies and almost 20 years of use in Brazil and Japan appear to support the safety of stevia extracts. Stevia rebaudiana extracts are used in weight-loss programmers due to their capacity to decrease cravings for sweet and fatty foods, as well as to treat diseases, hypoglycemia, candidiasis, skin abrasions, and inhibiting growth and reproduction of bacteria-like plaque (Gregersen S, Jeppesen PB, et al., 2004).

It does not affect blood sugar levels hence safe for diabetics. It also has anti-inflammatory and antioxidant properties. Further research is needed because some areas have not yet been fully clarified. In particular, there are few studies on its hepatoprotective action, hence the current investigation was carried out to formulate silver nanoparticles of stevia leaf extract against rat liver damage caused by paracetamol induce hepatotoxicity. Hence silver nanoparticle gives good protection against paracetamol toxicity and increases in oxidative stress and subsequent saturation of the

glucuronidation and sulfation pathways of hepatic elimination are the main causes of acetaminophen-induced hepatotoxicity.

As a result, more APAP is converted by cytochrome P450 (CYP) mediated oxidases into a highly reactive intermediate called N-acetyl-p-benzoquinone imines (NAPQI), which is toxic to the liver (Jaeschke et.al., 2003). When GSH is finally depleted, NAPQI is free to form APAP cysteine adducts with necessary hepatocellular proteins, which leads to the development of hepatic necrosis (Adam et.al, 2016).

Hepatocytes that are in the process of dying release hepatic enzymes into the bloodstream and exhibit changes in the activity of other metabolic enzymes and cellular integrity (Ding et al., 2016). Therefore, the amount of NAPQI produced and the availability of hepatic GSH for the detoxification of this toxic metabolite determine the toxicity of APAP.

Because of their features, including reduced toxicity, enhanced biodegradability, and bioavailability, silver nanoparticles (AgNPs) have been used for a variety of medicinal reasons. (Shanmuganathan R et al., 2018) Because of their small size, they are known to interact with biological systems without difficulty. AgNPs can be produced in a variety of ways, but green synthesis is now the most popular method since it has many advantages over chemical synthesis.

2. EXPERIMENTAL METHODOLOGY

2.1 Sample collection and authentication –

Plant was collected and authenticates to department of botany Dr. Harisingh GourVishwavidyalaya Sagar M.P with herbarium no. BOT/H/12/206/01.

Fig.1 Stevia leaves

2.2 Determination of extractive values

2.2.1Determination of Alcohol soluble extractive values

Macerate 10 gm of the air-dried drug, coarsely powdered, with 100ml of 90% ethanol in a stopper conical flask for 24 hrs., shaking the contents frequently during the first 6 hrs., thereafter filter rapidly taking precautions against loss

of ethanol. Evaporate 25ml of the filtrate to dryness in a water bath in a tarred flat bottom Petri-plate. Dry at 105°C for 1 hr. in a hot air oven, cool in desiccators and weigh repeat the process till the concurrent weight is obtained, and calculate the percentage of alcohol soluble extractive with reference to the air-dried drug.

$$\boldsymbol{AlcoholsolubleExtractive}\ \% = \boldsymbol{B} - \boldsymbol{C}/\ \boldsymbol{A} \times 4 \times \mathbf{1}00$$

Where, weight of empty crucible = C, weight of plant material = A, weight of Petri-plate + residue = B, weight of residue = B-C (Vijay Danapur et.al 2018)

2.2.2 Determination of Water-soluble extractive values

Macerate 10g of air-dried drug, coarsely powdered, with 100 ml of chloroform water in a Stoppard conical flask for 24 hrs., shaking the contents frequently during the first 6 hours. Thereafter filter rapidly by decanting the water extract. Evaporate the 25 ml of the filtrate to dryness on a water bath in tarred flat bottom Petri-plate or shallow dish. Add 2 ml of alcohol to the dry residue shake the contents and dry again in a water bath. Dry at 1050 C for 1 hr. in a hot air oven & cool in a desiccator for 30 minutes and weigh. Repeat the process till the concurrent weight is obtained; now calculate the percentage of water-soluble extractive of the air-dried drug.

$$\boldsymbol{watersolubleExtractive}\ \% = \boldsymbol{B} - \boldsymbol{C}/\ \boldsymbol{A} \times \mathbf{4} \times \mathbf{10}0$$

Where, the weight of the empty crucible = C, the weight of plant material = A, the weight of Petri-plate + residue = B, and the weight of the extractive = BC. (Vijay Danapur et.al, 2018)

2.3 Determination of ash values

Ash value is determined to check the quality and purity of a drug, especially a powdered drug. It is determined by the Muffle furnace.

2.3.1 Determination of total ash

Two grams of the powdered drug were incinerated in a sintered silica crucible by gradually increasing heat up to 4500C until the drug is free from carbon and then cooled. This ash was kept in a desiccator for 15-20 min. and weighed using an electronic balance, India, and noted down the readings (Raghunathan et.al, 1976)

$$\boldsymbol{Ash}\ \% = \boldsymbol{B} - \boldsymbol{C}\ /\boldsymbol{A} \times \mathbf{10}0$$

Where, the weight of the empty crucible = C, the weight of plant material = A, the weight of crucible + ash = B, and the weight of ash = B-C.

2.3.2 Determination of Acid soluble ash

The total ash obtained was boiled for 15 min. in 25 ml of 25% hydrochloric acid and filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in

desiccators for 15 min. The residue was weighed in a named electronic balance and the acid-soluble ash was calculated using the formula.

$$\text{A}\boldsymbol{cidinsolubleash}\ \% = \boldsymbol{B} - \boldsymbol{C}/\ \boldsymbol{A} \times \mathbf{10}0$$

Where, the weight of the empty crucible = C, the weight of plant material = A, the weight crucible + ash = B, and the weight of ash = B-C.

2.4 Phytochemical Screening

2.4.1 Test for alkaloids

- **Dragendroff's test:** A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to 2 ml of each extract. A turbid orange/orange red precipitate was seen to be an orange-red of alkaloids.
- **Wagner's test:** 1-2 ml of extracts were mixed with a few drops of Wagner's reagent to detect the presence of alkaloids. The test is positive when there is a brown flocculant or reddish-brown precipitate.
- **Hager's test:** Hager's reagent applied with extract produced a yellow-colored precipitate, confirming the presence of alkaloids.

2.4.2 Tests for phenolics compounds

- 1% potassium ferrocyanide and 1% ferric chloride solution were combined in equal parts. To 2 ml of extract, 3 drops of this freshly made combination were added. Positive results reveal the development of a bluish-green hue.

2.4.3 Test for tannins

- **Ferric chloride reagent test:** 3–4 drops of a 5% ferric chloride solution was added to one of the Extracts and left to react. Tannin is present when a strong green, greenish-brownish-green color forms.
- **Potassium dichromate test:** One milliliter of 10% aqueous potassium dichromate solution was allowed to be added to each extract solution. The development of a brownish-yellow precipitate indicated the presence of tannins.
- **Gelatin test:** When 1% sodium chloride-containing gelatin solution is added to the extract, a white precipitate forms, indicating the presence of tannin.

2.4.4 Tests for Flavonoids

- **Alkaline reagent test:** The presence of Flavonoids was determined by adding 1 ml of a 10% sodium hydroxide solution to the test sample, which resulted in a bright yellow color that turned colorless when diluted acid was added.
- **Lead acetate test:** Extracts were exposed to a few drops of a 10% lead acetate solution in order to detect the presence of Flavonoids through the production of a yellow-colored precipitate.

2.4.5 Test for steroids

- **Salkowki's test:** A lower layer was created by carefully adding strong sulfuric acid to the test tube wall after adding 1 ml of chloroform to 2 ml of each extract. At the interface, a reddish-brown ring that appeared right away

revealed the presence of steroids.

2.4.6 Test for saponins

- **Froth test:** After shaking 5 ml of extract briskly for 30 seconds and letting it stand for 10 minutes, it was looked for the stable, persistent foam that indicates the presence of saponins.

2.4.7 Triterpenes

- The ethanolic extract of S. rebaudiana has demonstrated the presence of Triterpenes by passing the Salkowki's and Leiberman-Buchardt tests with flying colors of golden yellow after vigorous shaking and red ring, respectively.

3. Preformulation study

i. Determination of Melting Point - The melting point of stevia was measured using DSC apparatus. The observed melting point data was 192°C respectively.

ii. Solubility profile -The solubility of the stevia leaves extract powder in ethanol, water, chloroform, and diethyl ether Buffer PH 7 was examined after 1mg was placed in a test tube.

iii. Determination of λ-max and preparation of calibration curve of stevia rebaudiana extract-:

10 mg of stevia leaves extract was dissolved in 10 ml of distilled water to give a concentration of 1 mg/ml. Then UV-visible spectra were taken in the range of 200- 400 nm using water as blank (I. Johnson et.al, 2015). From this solution, 1 ml was diluted with 1 ml of distilled water. Further dilutions are made to get the concentration (20-100 µg/ml). Then absorbance of each solution was measured at 210 nm using water as a blank (Mukesh S. Sikarwar et.al, 2008).

iv. Estimation of secondary metabolites: Stevioside and rebaudioside a by HPTLC method

For the simultaneous measurement of stevioside and rebaudioside A, a high-performance thin layer chromatographic (HPTLC) approach was created: Acetone, ethyl acetate, and water (5:4:1, v/v/v) were used as the solvent solution on pre-coated silica gel 60 F254 TLC plates to produce the separation. After spraying with anisaldehyde sulphuric acid as a detecting reagent, the densitometric measurement of stevia glycosides was performed at wavelength 360 nm in absorption mode. Stevioside and rebaudioside A well-resolved peaks were found at Rf values of 0.31± 0.02 and 0.21± 0.02 respectively. (Karishma Chester et.al 2012)

3.1 Preparation of AgNPs

Preparation of 1Mm Concentration of Silver Nitrate Solution- The molar mass of silver nitrate (AgNO3) is 169.87g/mole. A one-mile molar solution of silver nitrate was prepared by using the following formulae,

$$\frac{\text{Molecular weight} \times \text{Request Milli molar} \times \text{Request Volume}}{1000}$$

For the preparation of 100ml of solution 0.017g of silver nitrate was dissolved in distilled water. (Ajithadas aruna et.al, 2014)

3.2 Green synthesis of silver nanoparticle

A mortar and pestle were used to ground the dried leaves into a fine powder. In 100 ml of double-distilled water, 10g of freshly made dry leaf powder was suspended, and the mixture was heated for 15 minutes at 40°C. The solution was then put through a Whatman No. 1 filter paper filtering process and utilized as a stock solution for the subsequent production of silver nanoparticles. A 90ml amount of distilled water was used to prepare a silver nitrate solution with a 1mm concentration. The 90 ml of silver nitrate solution was then added to the 10 ml of leaf extract. After the 24-hour period of darkness-induced incubation. A dark brown color solution was created from the colorless reaction mixture. The color change was occurring because of the presence of the bioactive molecules (Phytochemicals) present in the stevia leaves extract was reduce the silver metal ions into silver nanoparticles The intensity of the color change was increased by the incubation period at different intervals for the nanoparticle's synthesis. (N. Siddharthan, et al.,2019)

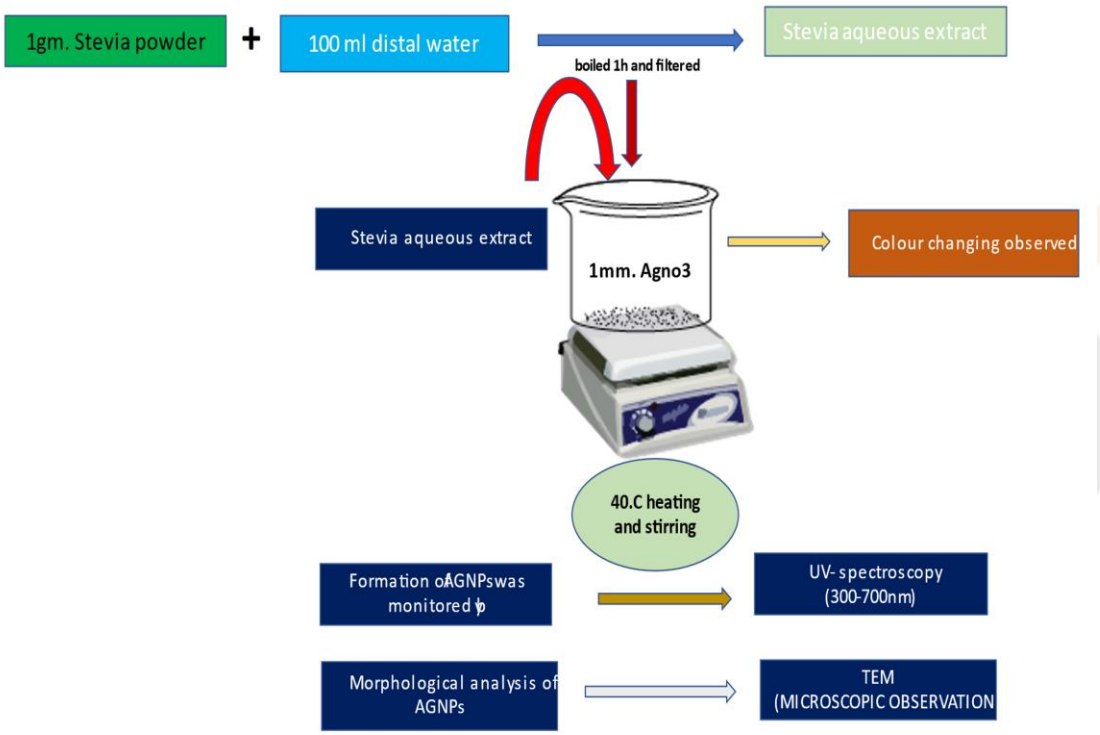


Fig2. Preparation of AgNPs

3.3 Separation of Silver Nanoparticles

By centrifuging the synthesized stevia leaves extract silver nanoparticles at 5000 rpm for 15 minutes, they were separated. The pellets were collected and stored while the supernatant liquid was thrown away. (Ajithadas Aruna et.al. 2014)

3.4 Characterization of synthesized Silver nanoparticle of stevia leaf extract

3.4.1 Visual Examination

The visual foundation is used as the initial validation of the synthesized stevia leaves extract silver nanoparticles. Stevia leaves extract and silver nitrate solution was colored differently throughout time (I. Johnson, H. Joy Prabhu, 2015).

3.4.2 UV- spectra analysis

Using spectroscopic examination at 300-700 nm, the bio-reduction of Ag+ ions in the aqueous extracts and the production of AgNPs were observed. (Abd El Raouf N, Hozyen W 2017)

3.4.3 TEM

Using transmission electron microscopic (TEM) examination, the size, shape, and AgNPs condition were monitored morphologically. AgNPs sample in aqueous form was put onto a copper grid that has been carbon-coated and allowed to completely dry at room temperature for 12 hours and detailed microscopy images were observed.

3.4.4 Drug Entrapment

In distilled water, 10 mg of stevia leaf extract silver nanoparticles were dissolved. After centrifugation, UV Spectrophotometry was used to calculate the amount of drug contained in the supernatant (Renu Tiruwa et.al 2015).

3.4.5 Determination of Particle size

The mean size of the nanoparticles was determined using a Zetasizer Nano series (Malvern Instruments, England). Samples of all nanoparticles were dispersed in distilled water just prior to analyses. According to the definition of the nanosphere, the size of nanospheres should be between 1-1000 nm. In all formulations, the mean particle size of the prepared nano spheres ranged from (500-800.) nm. So, it can be said that the prepared formulations were nanospheres in terms of their particle size.

3.4.6 Determination of Zeta potential

The stability of AgNPs is significantly and easily measured by the zeta potential. The size of the zeta potential represents the strength of electrostatic attraction between nearby particles with identical charges in dispersion. For sufficiently tiny molecules and particles, a strong zeta potential will offer stability.

3.4.7 DSC of prepared AgNPs

3.4.8 In vitro drug release study

Silver nanoparticles from stevia extract were studied for in-vitro drug release using the dialysis bag diffusion method. This approach made use of a Dialysis Membrane which has a molecular weight cutoff of 12000 to 14000. For around

30 minutes, a dialysis membrane was heated in boiling water to activate it. The dialysis membrane was then sealed at both ends and included the manufactured stevia silver nanoparticles. It was then put into a beaker with 100 ccs of pH 7.4 phosphate buffer then beaker was then positioned over a magnetic stirrer with the rpm kept at 100. At certain intervals, samples (2 ml) were removed and replaced with equal volumes of fresh phosphate buffer at pH 7.4. The samples were examined following the appropriate dilutions at 206 nm, using a UV-Visible spectrophotometer. (Bohrer et al 2016).

5. In Vivo Study

Animals were randomly divided into five groups of six animals each. Group, I served as control, and group II was administered only paracetamol 2mg/kg body weight once only and served as a negative control. Group III-VI –V were intoxicated with APAP at a dose of 2g/kg p.o. once only. After then group III was treated with the standard drug it is silymarin 50mg/kg body weight. Group VI was treated with stevia leaf extract 200mg/kg body weight group V was treated with AgNPs of stevia extract animals all groups were sacrificed after 24h of the last treatment.

The study protocol was approved by the Ethical Committee for Animal Experiments, DR. Harisingh Gour university (Approval No. 379/CPSEA/IAEC/2021/24).

Table 1. Animal groups as divided and used in the study with planned treatments for 7 days

Group	Treatment
First	Control
Second	Paracetamol (negative control)
Third	Paracetamol+ silymarin (standard drug)
Fourth	Paracetamol+ Stevia extract
Five	Paracetamol+ AgNPs of stevia

- **Isolation of serum**

Blood was drawn from the retro-orbital venous sinus (Riley, 1960). After centrifuging the blood for 15 minutes at 2000 rpm after allowing it to stand at room temperature for 30 minutes, serum was extracted and kept at -20 C

- **Blood biochemistry**

For the estimation of liver function tests (LFT) markers like serum transaminases AST and ALT, ALP, GSH, MDA, and bilirubin serum were used (Reitman and Frankel, 1957). The following parameters were assessed using diagnostic kits and an auto-analyzer (kavya pathology Bhopal M.P).

- **Histopathology research**

A little part of each animal's liver from all five groups was preserved in 10% w/v buffered formal saline (pH 7.4). After that, the paraffin section was produced and dyed using the use of the dye hemotoxylin-eosin to detect liver damage.

6. RESULT AND DISCUSSION

6.1 Physical appearance: The physical properties of crude drugs stevia rebaudiana leaves extract are described in the below table

Table 1. Physical properties of S. rebaudiana

S.NO.	Physical Characteristics	S. rebaudiana
1.	Color	Green
2.	Sensation	Smooth
3.	Taste	Sweet
4.	Oily stain	No

6.2 Determination of extractive values

The water-soluble extractive value and Alcohol soluble extractive value of the drugs was determined and reported below in the table

Table 2. Extractive value of S. rebaudiana

S.NO.	Extractive values %w/w	S. rebaudiana%
1.	Water soluble Extractive value	**12.34**
2.	Alcohol Soluble Extractive value	**7.14**

6.3 Determination of ash values

Ash value such as Total Ash, Acid soluble ash was determined using a Muffle furnace. The Ash values of S. rebaudiana reported below in the table

Table 3. Ash value of S. rebaudiana

S.NO.	Ash values % w/w	S. rebaudiana%
1.	Total Ash	**8.50**
2.	Acid soluble Ash	**1.25**

7. PREFORMULATION STUDY

7.1 Determination of melting point- The melting point of stevia was measured using DSC apparatus the observed melting point data was 185-195°C respectively

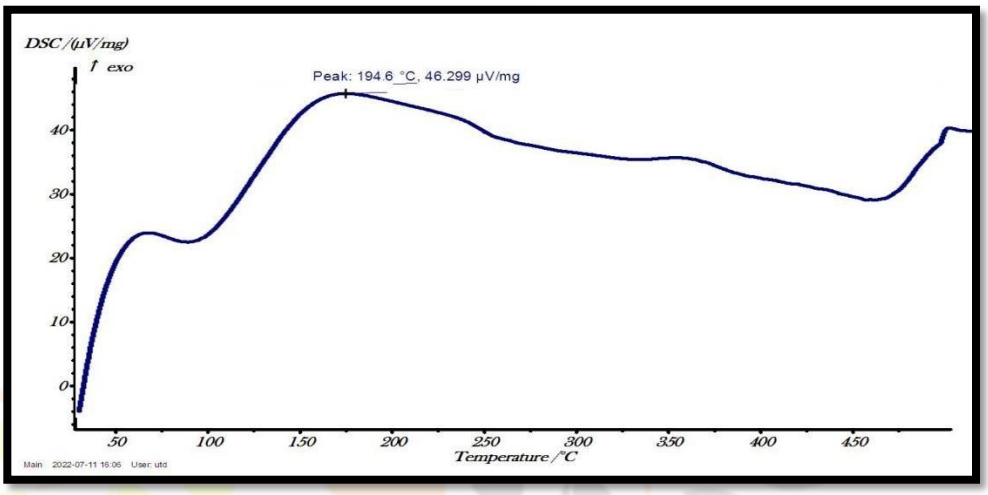


Fig.3 DSC of Stevia leaves extract

7.2 Solubility profile

Table 4. solubility profile of S. rebaudiana

S.NO.	Solvent	Result
01	Water	Soluble
02	Methanol	soluble
03	Ethanol	soluble
04	Acetonitrile	Soluble
05	Buffer 7.4PH	soluble

7.3 Preliminary Phytochemical tests

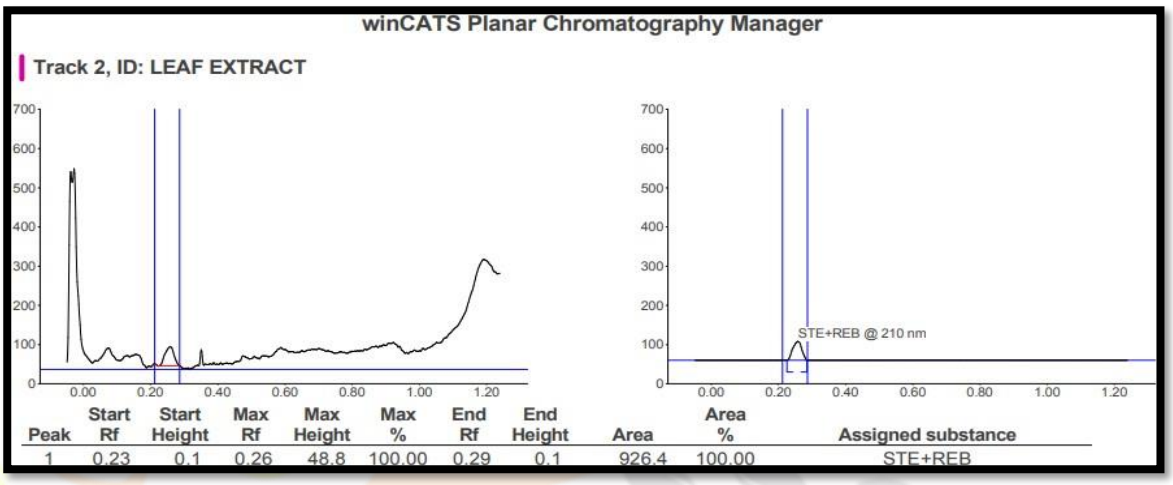
Table 5. Phytochemical test of S. rebaudiana

S.NO	Chemical test	Result
1	**Test for phenols**	
	➢ Phenol test	+
2	**Test for Flavonoids**	
	➢ Shinoda test	+
	➢ Flavonoids test	+
3	**Test for steroids**	
	➢ Salkowki's test	+
	➢ Leiberman-Buchardt test	+
4	**Test for Triterpenes**	
	➢ Salkowki's test	+
	➢ Leiberman-Buchardt test	+
5	**Test for lactones**	
	➢ Feigels test	+
	➢ Baljet test	+
6	**Test for tannins**	
	➢ Tannin test	+
	➢ Gelatin test	+
7	**Test for alkaloids**	
	➢ Mayer's test	+
	➢ Wagner's test	+
	➢ Dragendroff's test	+

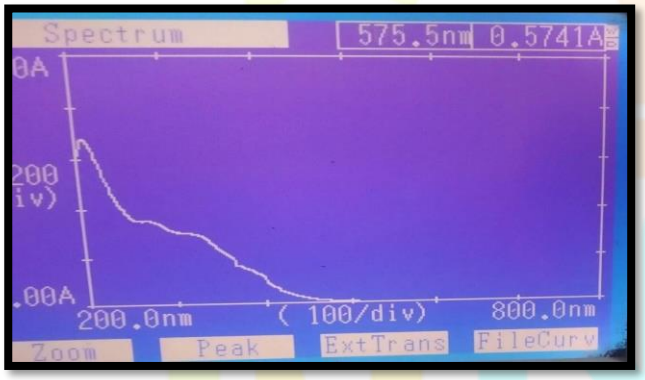
7.4 Estimation of secondary metabolites: Stevioside and rebaudioside by HPTLC

S.NO.	Requirement	Result
1.	Solvent system	Acetone, ethyl acetate, and water (5:4:1, v/v/v)
2.	TLC plate	Pre-coated silica gel 60 F254
3.	Spraying reagent	anisaldehyde sulphuric
4.	Rf value of stevioside	-0.26
5.	Rf value of rebaudiosides	-0.26

Fig.4 HPTLC Chromatograph of stevia extract



7.5 Determination of λmax and calibration curve



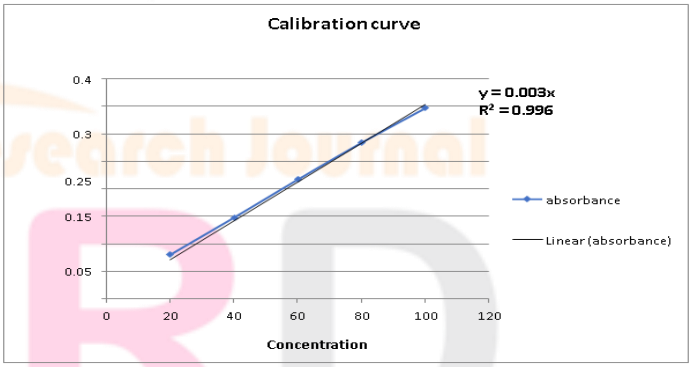


Fig.5 calibration curve

7.6CHARACTERIZATION OF SILVER NANOPARTICLE

7.6.1Visual Examination: The visual foundation is used as the initial validation of the synthesized stevia leaves extract silver nanoparticles. Stevia leaves extract and silver nitrate solution was colored differently throughout time (I. Johnson, et al., 2015).

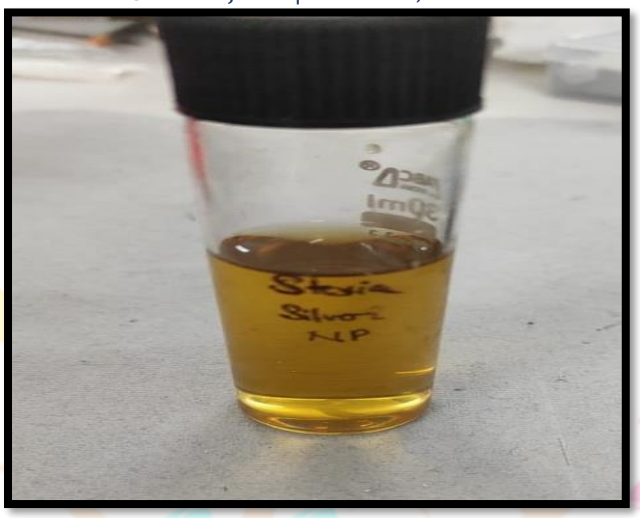


Fig.6 Color change of Synthesized AgNPs

7.6.2 Analysis using an ultraviolet (UV)-visible spectrophotometer

Proved that AgNPs bio-synthesized. The results showed a peak at 435 nm in the reaction mixture's absorption spectrum at various wavelengths between 400 and 500 nm

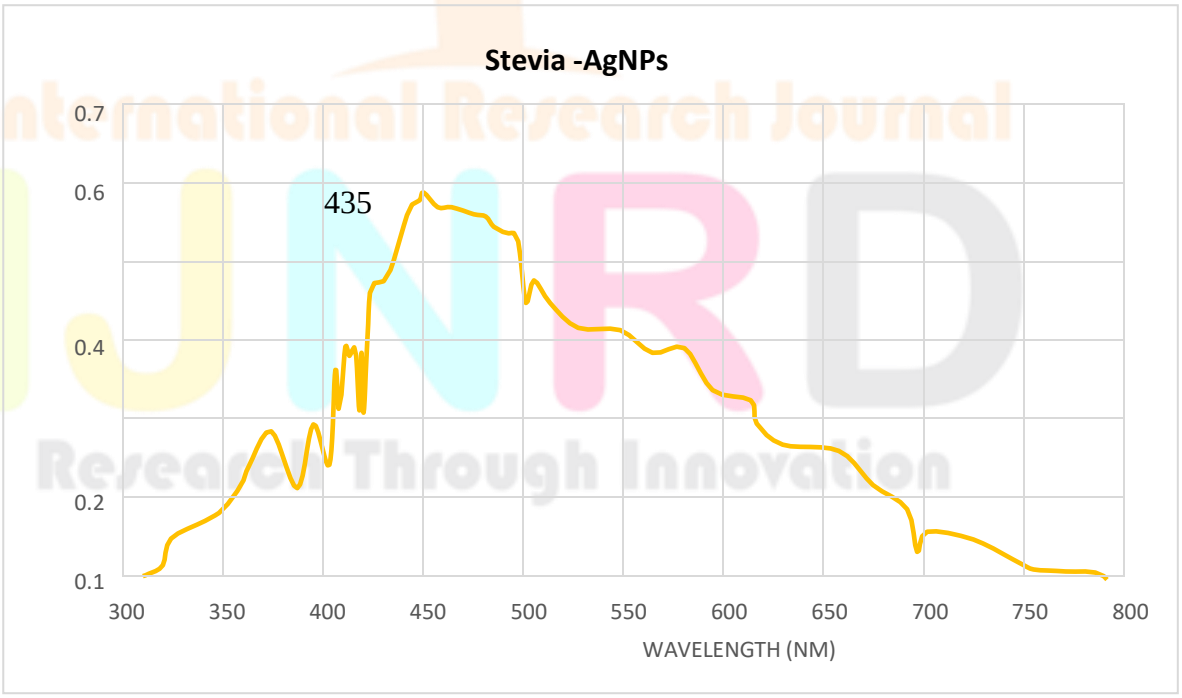


Fig.7 Analysis using an ultraviolet (UV)-visible spectrophotometer

7.6.3 TEM

The size and morphology of the silver was studied via transmission electron microscopy (TEM).

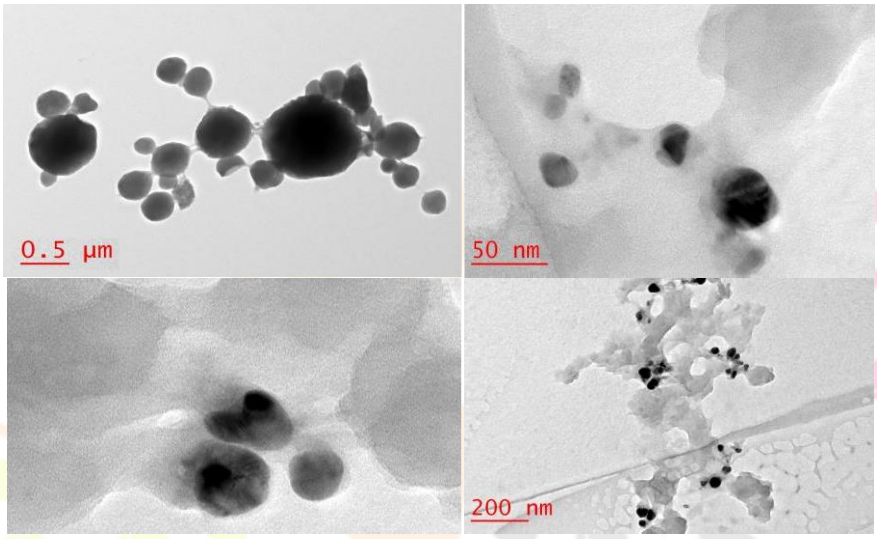


Fig.8 TEM image of stevia extract Ag-NPs

7.6.4 Determination of particle size and Zeta potential

The stability of AgNPs is significantly and easily measured by the zeta potential. The size of the zeta potential represents the strength of electrostatic attraction between nearby particles with identical charges in dispersion. For sufficiently tiny molecules and particles, a strong zeta potential will offer stability.

1.	**Diameter (d)**	**137.5 (nm)**
2.	**Polydispersity Index (P.D.I.)**	**0.226**
3.	**Zeta potential**	**-33.76**

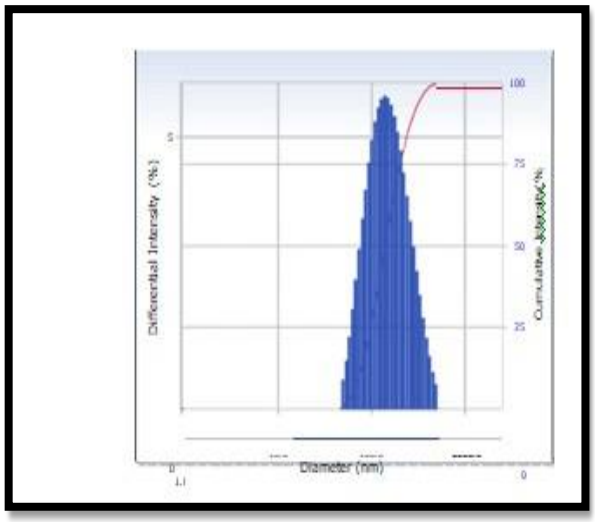


Fig.9 Particle size of AgNPs

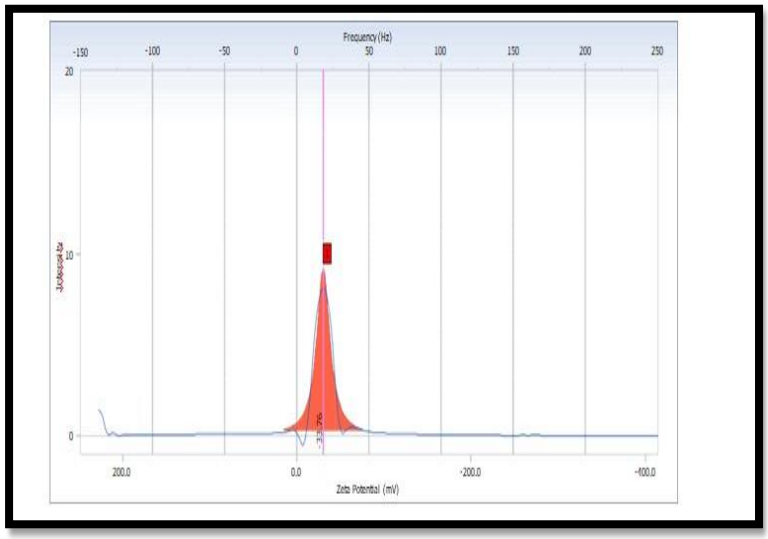


Fig.10 Zeta potential of AgNPs

7.6.5 XRD (X-ray diffraction)

The physical state of stevia extract AgNPs was examined by XRD analysis. The diffractograms of AgNPs are shown in fig. This demonstrates the high-intensity diffraction peaks for pure drugs, depicting their crystalline nature.

7.6.6 DSC (Differential scanning calorimetry)

A sharp endothermic peak at 194.6°C was obtained in the DSC curve of stevia extract, demonstrating its crystallinity and melting point. There was no chemical interaction seen between pure stevia extract and silver ion in the DSC curve of AgNPs, as the characteristic endothermic peak of stevia (194.6 °C) and AgNPs (192.9 °C) appeared separately in the graph. In addition, the thermogram of AgNPs showed an exothermic peak around 192.9 °C which corresponds to the reduction of the Ag ion of silver nitrate. These results are further confirmed with XRD analysis

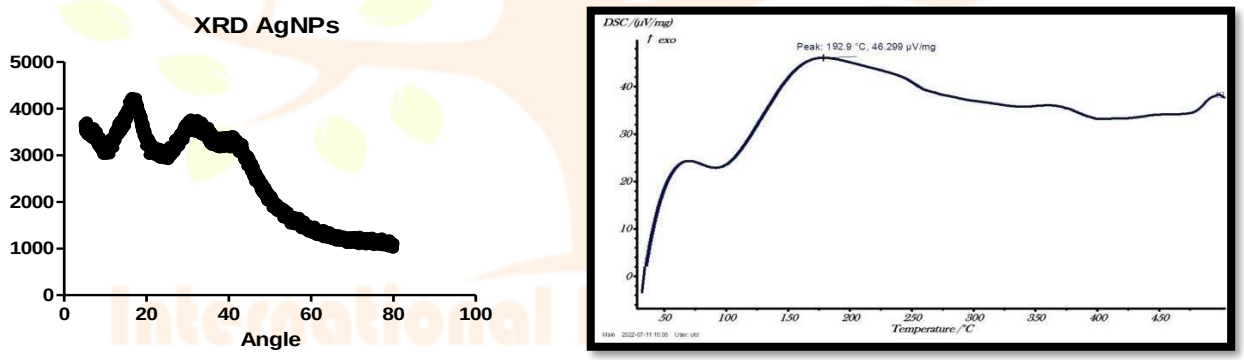


Fig.11and 12 DSC (Differential scanning calorimetry) and X-ray diffraction of prepared AgNPs

8. In vitro drug release study

Dialysis bag diffusion research was done to examine in-vitro drug release. We discovered the amount of medication released at various time intervals (1h, 2h, 3h, 4h, 5, 6h, and 7h). The diffusion dialysis technique is depicted in Figure.

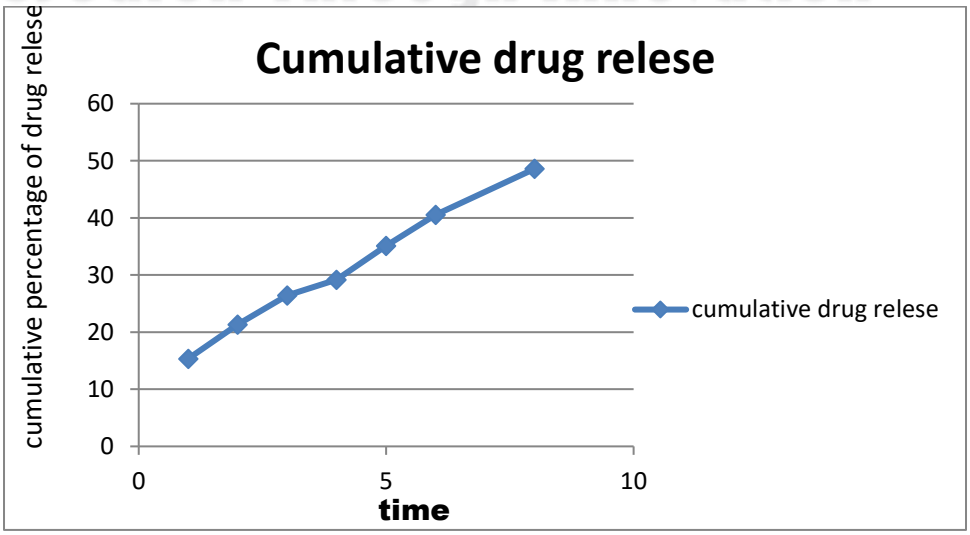


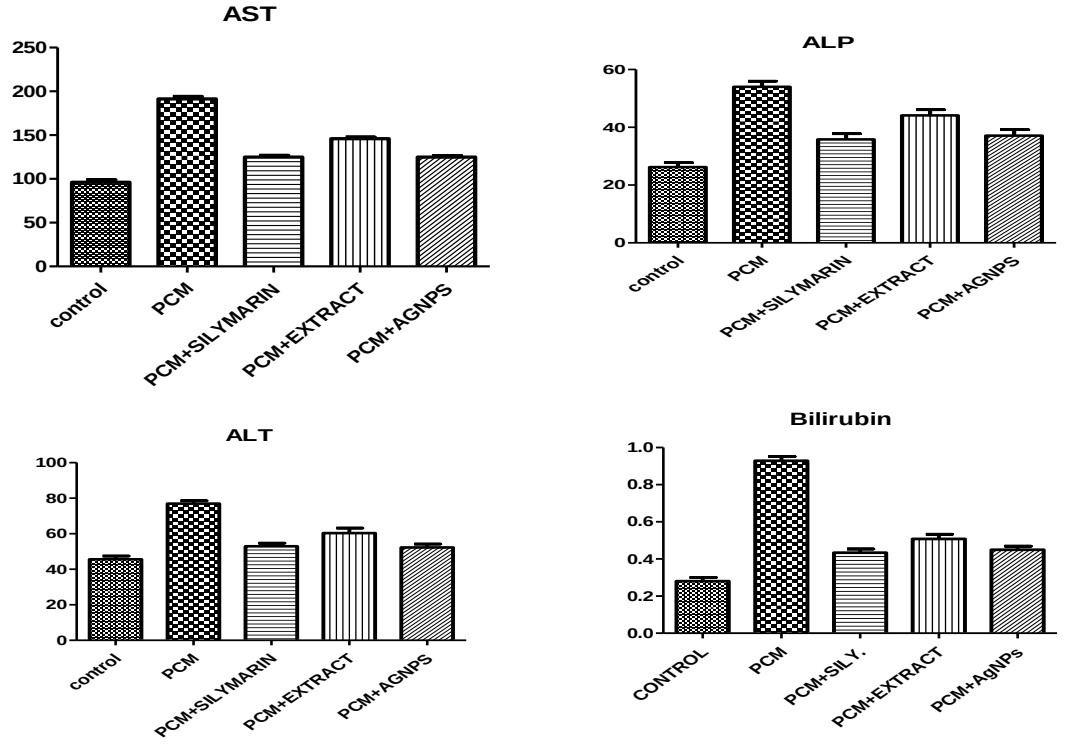
Fig.13 In vitro drug release study

9. In vivo study

Table 7. Effect of AgNPs on liver enzymes of rats intoxicated with paracetamol

Group	AST	ALT	ALP	Bilirubin
Control	96.07 ± 2.8	45.59 ± 1.8	26.2 ± 1.5	0.2800 ± 0.02
PCM	191.3 ± 2.7	77.00 ± 1.5	54.03 ± 1.9	0.9283 ± 0.02
PCM+Silymarin	124.9 ± 1.9	52.94 ± 1.7	35.83 ± 1.9	0.4333 ± 0.02
PCM+Extract	145.8 ± 1.6	60.35 ± 2.8	44.14 ± 1.9	0.5083 ± 0.02
PCM+AgNPs	124.9 ± 1.6	52.28 ± 1.9	37.11 ± 2.0	0.4500 ± 0.01

Showed that AST, ALT, and ALP, indicators of liver enzyme activity, and bilirubin were significantly elevated in Paracetamol-administered rats compared to controls, but treatment of rats with stevia extract -AgNPs and paracetamol caused those markers to significantly decrease compared to paracetamol rat



8. Table Effect of AgNPs on the anti-oxidant enzymes with intoxicated paracetamol

Group	CAT	SOD	GPx	GSH	MDA
Control	8.7 ± 0.14	16.12 ± 1.3	217.2 ± 1.9	54.8 ± 1.1	11.3±0.8
PCM	3.6± 0.17	5.7 ± 0.6	97.67 ± 2.4	27.0 ± 0.9	27.9 ± 0.8
PCM+Silymarin	6.9 ± 0.2	14.01 ± 0.7	188.5 ± 3.6	53.7 ± 1.3	12.3 ± 0.6
PCM+Extract	5.6 ± 0.15	11.2 ± 0.8	170.8 ± 2.6	46.8 ± 1.3	20.2 ± 0.4
PCM+AgNPs	6.9 ± 0.2	13.9 ± 0.8	170.8 ± 2.6	52.4 ± 0.9	12.8 ± 0.4

When compared to control rats, paracetamol poisoning caused hepatic tissues to have significantly higher levels of MDA and lower levels of GSH as well as lower SOD, and CAT activity. Rats given paracetamol intoxication were treated with stevia extract-AgNPs, which significantly reduced MDA and activity while remarkably increasing GSH levels and antioxidantenzymes [Table]

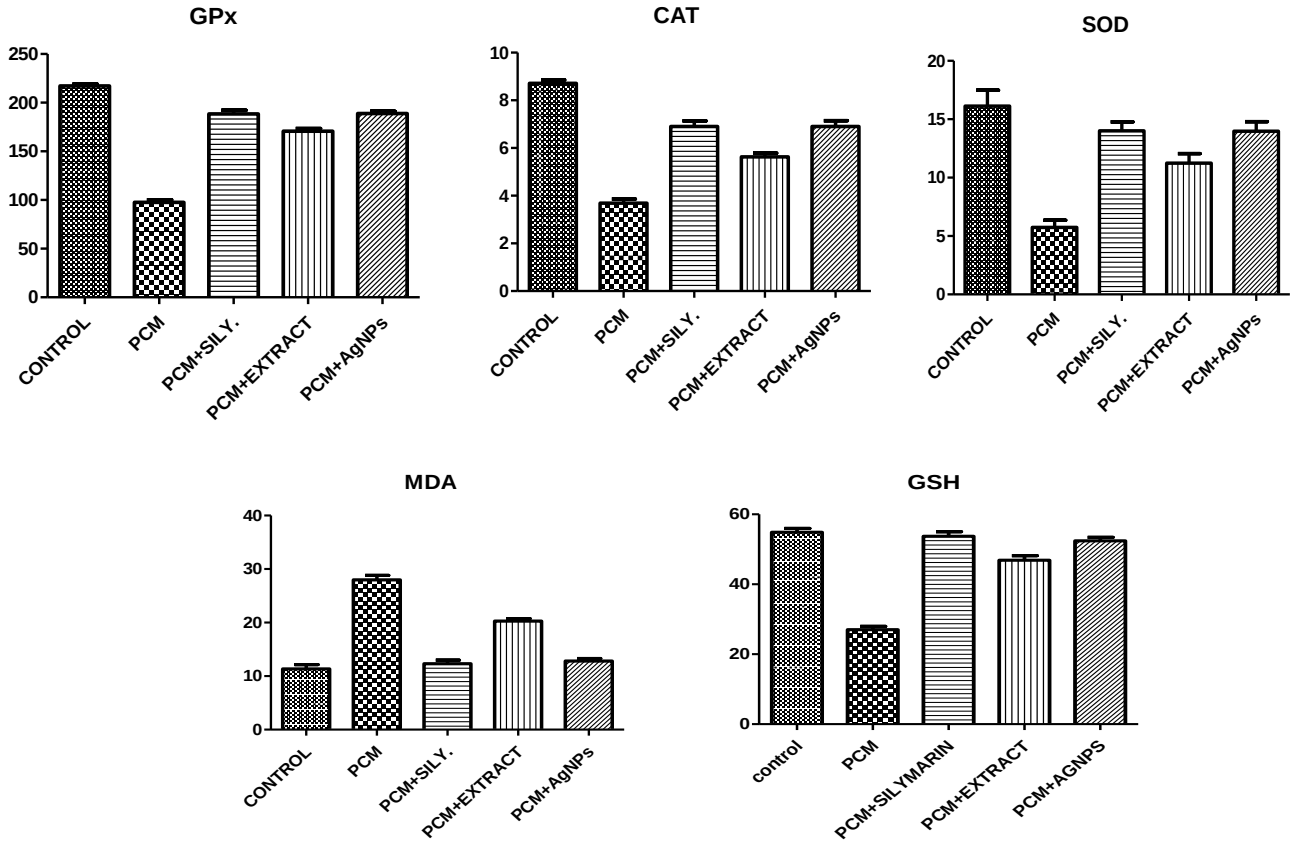


Figure: (A) Glutathione peroxidize (B) Catalyse (C) Superoxide Dismutase (D) Malondialdehyde (E) Reduce Glutathione

9.1 Histological Examination

The tissue samples were taken rapidly from each rat and fixed in 10% formalin for histopathological study. All the samples were dehydrated in ascending grades of ethanol, cleared in butanol, and embedded in parablast. Sections of 5–6 thick sections were obtained and stained with the following stains: Haemotoxylin and eosin staining for general histological study.

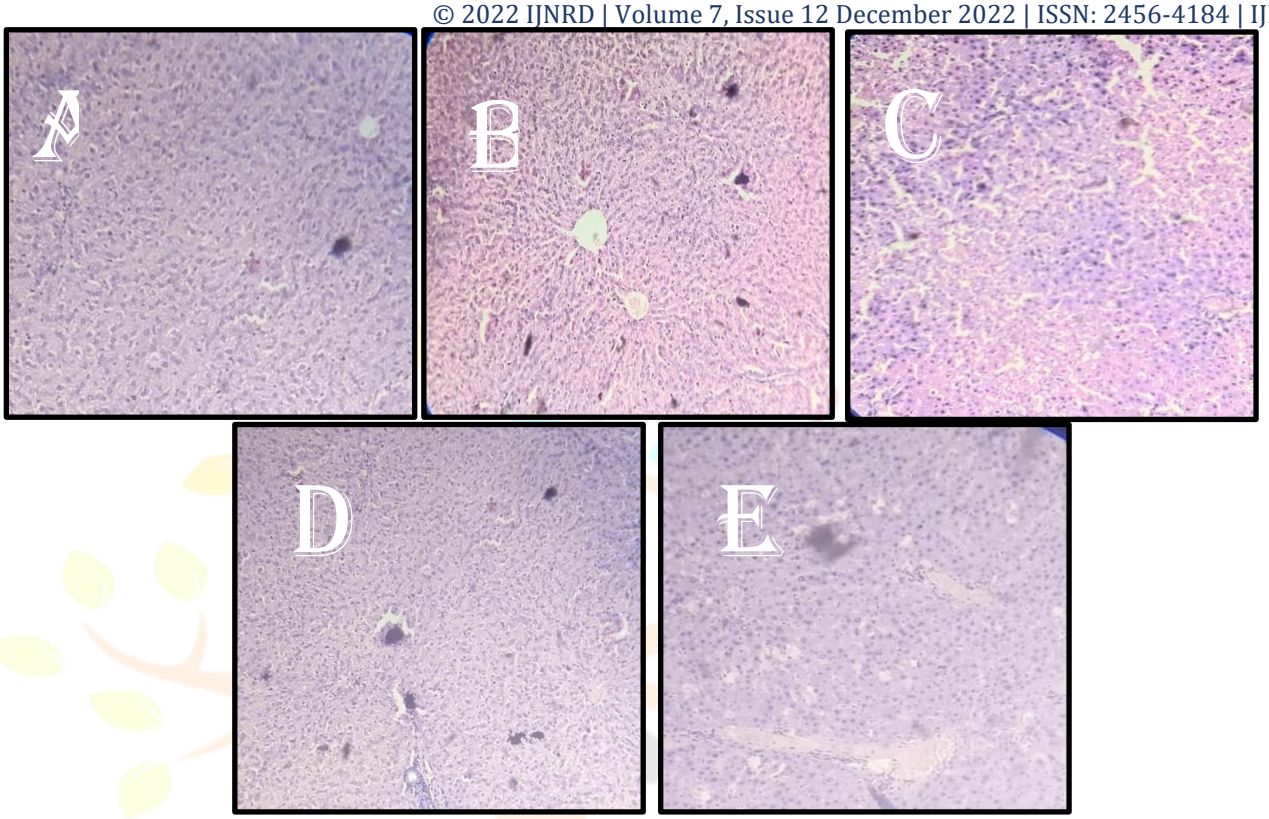


Figure: Histological structure of different group rats' liver: (A) Normal rat liver. (B) Intoxicated with paracetamol rat liver. (C) Paracetamol was intoxicated and treated with silymarin 100mg/kg B.W.(D) Paracetamol was intoxicated with stevia extract 200mg/kg. (E) Paracetamol intoxicated treated with Stevia AgNPs 200mg/kg

10. Summary and Conclusion

. Encapsulation into silver nanoparticles effectively increases the activity of herbal extracts with promising antihepatoprotective properties. The results of this study revealed that stevia extract can be efficiently encapsulated into NPs and Ag-NPs exhibited sustained release at various pH. Further, Ag-NPs demonstrated noticeable antioxidant activity owing to the phenolic and flavonoid contents of the stevia extract. Encapsulation of stevia extract into NPs improved the antihepatoprotective activity of the extract in a synergistic manner. They are over-activation revealed another approach to liver injury due to the influence of any toxicant. Overall, our results suggest that Ag-NPs are a promising herbal-based stevia delivery system for use as an anti-hepatoprotective therapy.

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