DMBA-induced breast tumor-associated anti-inflammatory and antioxidant activity of green synthesis of silver nanoparticle of *Russelia equisetiformis* flower extract

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ABSTRACT

Background: Nanotechnology is the specialty associated with material science and biology, rather than a particular field. It entails the method of particles at the nanoscale called Nanoparticles, wherein they have control over bulk macroscopic properties of the identical material. The "drug nanocarrier," silver possesses strong antibacterial, antioxidant, and anti-cancer as well as anti-inflammatory and antioxidant properties. As the medicinal plant *Russelia equisetiformis* flower possesses a lot of phytochemicals, this study was conducted to assess the anti-inflammatory and antioxidant activity of silver nanoparticles (AgNPs) reinforced with *Russelia equisetiformis* flower extract Materials and Methods: Anti-inflammatory activity and antioxidant activity of AgNPs reinforced with *Russelia equisetiformis* flower extract were assessed by IL- and IL-10 serum level measurement after induction DMBA in the mammary gland. anti-oxidative activity measurement by assessment of SOD, LPO, GSH, and catalase level.100 and 50m mg dose was selected for study as per OECD guideline, and nanoparticles were characterized by SEM and EDX methods The values for the anti-inflammatory property of nanoparticles with *Russelia equisetiformis* flower extract greater than only *Russelia equisetiformis* extract which significant with Negative control group The values for an antioxidant property of nanoparticles were found to be higher than the DMBA controlled group but lower than tamoxifen group AgNPs reinforced with Russelia equisetiformis flower extract have potential as an anti-inflammatory and antioxidant agent and can be used as an alternative to commercially available products.

Keywords: Anti-inflammatory, antioxidant, silver nanoparticles, *Russelia equisetiformis*
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1. INTRODUCTION

Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer with unknown Etiology and generally poor outcome. It is characterized by diffuse edema and redness (erythema), although either the disease itself or case definitions have varied over time and place, confounding temporal trends and geographic variations [1]. Researchers have been interested in nanomaterials because of their size and potential applications in a variety of fields of

human-beneficial science, particularly noble metals. It is possible to create metal nanoparticles utilizing environmentally friendly, commercially feasible, and promote synthesis of silver nanoparticles due to their size and shape. In the synthesis and production of theragnostic silver nanoparticles (NPs), which have been produced utilizing a variety of materials, plant materials are of special interest. However, chemically created nanoparticles have several disadvantages in terms of cost, toxicity, and efficiency. To address the limitations of conventional synthesis syntheses, such as physical and synthetic methods, a plant-mediated integration of metallic nanoparticles has been devised. Nanomaterials are complex tools because of their adjustable properties. To get over the limitations of conventional synthesis, such as physical and synthetic methods, a plant-mediated integration of metallic nanoparticles has been created. Nanomaterials They are complex tools in the biomedical platform thanks to their customizable properties, which are particularly useful for creating novel therapies and diagnostics for cancer, neurological, and other chronic illnesses [2]. Numerous studies have demonstrated the positive benefits secondary chemicals, which are found in many plants, have on human health. These effects include cardiovascular protection, anti-cancer activity, antinociceptive activity, and anti-inflammatory properties. The public is becoming more and more aware of the issues related to the overuse and overprescribing of synthetic anti-inflammatory medications. Many herbal remedies are available over-the-counter from herbal suppliers and natural-food stores. Self-medication with these substances is commonplace, and HERE is growing interest in using plant chemicals to alleviate inflammation [3]. Due to their extensive spectrum of pharmacological effects, natural compounds originating from plants, such as flavonoids, sterols, polyphenols, alkaloids, tannins, and terpenes, have gained relevance in recent years. Researchers are now evaluating the biological properties these naturally occurring compounds generated from plants possess. 3 Finding natural remedies with fewer adverse effects and less risk for addiction, like opioids, might be crucial in the treatment of inflammation and pain conditions.

2. MATERIALS AND METHODS

Reagents and chemicals

The DMBA, also known as 7,12-dimethylbenz[a] anthracene, was created in theElisa CRP, and IL-6& IL-10 kit for biomarker test was ordered from Maxome Life Science in Bangalore, India, and was manufactured in the United States by Sigma-Aldrich. Throughout the procedure, analytical-grade chemicals were used. Unlike Merck, which provided the ethanol, LobChemical Pvt. Ltd. provided the silver nitrate (AgNO3, 99.8%). (Germany).Deionized and Milli-Q water was used during the whole study period[5].

Investigation of Phytochemicals

The presence of lipids, volatile oils, triterpenoids, tannins, alkaloids, phenols, flavonoids, anthraquinones, tannins, and saponin was investigated in a preliminary phytochemical investigation of *Russelia equisetiformis* flower ethanol extract [6].

Green synthesis of silver nanoparticles

In a round-bottom flask with a cooling condenser and a magnetic stir bar, the plant extract and the 0.2 M silver nitrite (AgNO3) solution were mixed to create the reaction mixture. The mixture was allowed to mix for three hours at 90 to 95 °C before being cooled to room temperature (the immediate color change was observed from light brown to dark brown, and HEREafter no further color change was observed even after 1 hour). Two hours after the combination had been given time to cool, it was centrifuged. The centrifugation was done at a speed of 8000 rpm at a temperature of room temperature. After three piles of washing with distilled water and a short period of being dried in an oven set to 70°C, black powder (HERE-AgNPs) was generated [7].

Characterization of HERE-AgNPs

Nanoparticle behaviour, biodistribution, safety, and efficacy are all highly influenced by their physicochemical properties. As a result, it is crucial to characterize silver nanoparticles (AgNPs) to assess the created particle's qualities. analytical techniques like energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM).To analysed nanomaterials and nanostructures, one of the most used instruments is the scanning electron microscope (SEM). A sample's surface morphology (texture) and chemical composition can be determined from the signals produced by electron-sample interactions Equine-like Russelia Using a drop of the suspension in a clean electric stub and letting the water entirely evaporate, nanoparticles were suspended in deionized water and utilized for SEM examination. quantitative compositional analysis and an elemental analysis Also useful is an energy-dispersive X-ray analyzer (EDX) [8].

Animals

All in vivo test subjects were provided courtesy of the Indore-based Acropolis Institute of Pharmaceutical Education and Research (1627/PO/Re/S/12/ CPCSEA). IAEC no. AIPER/IAEC/2021/002 of the Institutional Animal Ethical Committee gave its approval to the method. The CPCSEA standards and recommendations were followed for all animal investigations. Rats received unlimited amounts of food and water (standard pellets). After a seven-day acclimatization period, the experimental work with the rats got underway. Two rats were housed in each cage, and they were divided into two groups using a randomization process. Therapy (HERE and conventional) was provided to the treatment group, but not to the control group. For the rats in the experiment, a 12-hour light/dark cycle was used to maintain the mean ambient temperature of the animal housing at (24°C 2°C) [9].

Acute toxicity studies

The "Fixed Dose Method" of the OECD 423 guideline was used in the current investigation to determine acute toxicity. For each phase, three albino Wistar rats were chosen, and oral dosages of HERE extract and HERE-AgNPs were delivered to the rats in the following ratios: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. A judgment on the acute toxicity of the test substance and/or the animals' morbid condition may take two to four phases, depending on the death rate [10].

Experimental design and procedure

For this study, healthy female Wistar rats aged 56 to 60 days were taken and the average weight of the rats was 170 grams. as per the study design shown (table no 01), rats were randomized and divided into 6 groups Shown in table no 1. DMBA is given to all groups (Group II to VI) except the Vehicle Control Group (Group-I) and it is considered this group as the 0th day. The rats were palpated twice a week for the identification of breast cancers. During the whole experiment, all groups of rats were weighed 0th days intervals. after that, drug dosing is started when the tumor size reaches 0.5 cm. All rats were given the drug dose as per the study design from day 56th to 100 days. The blood sample was collected onthe 101st day aftercompletingthe doseand the levels of IL-6& IL-10 in serum were measured by ELISA kits according to the manufacturer'sinstructions [11].

Table-01			StudyDesign
S.No	Group No.	Group Name	Dose,drugs,andSchedule
1	Group- I	ControlGroup	Only1mLofsalinewasgiven.0th to 101 days
2	Group II	Negativecontrol	All rats Only received DMBA (S.C 50 mg/kg) on the 0th day and were induced by subcutaneous administration in 1 ml

			sunflower oil
3	Group III	OnlyExtract (HERE)	AllRatstreatedwithDMBA(similartogroupII)+ extract(HERE)100mg/kggivenorallyat51days from the 0th day
4	Group IV	HERE-AgNPs Lowdose	AllRatstreatedwithDMBA(similartogroupII)+nanoparticle(HERE-AgNPs)50mg/kg/daygiven orally at 51 days from the 0th day
5	Group V	HERE-AgNPs Highdose	AllRatstreatedwithDMBA(similartogroupII)+nanoparticle (HERE-AgNPs) 100 mg/kg given orally at 51 days from the 0th day
6	Group VI	Standard (Positivecontrol)	AllRatstreatedwithDMBA(similartogroupII)+ Tamoxifen(20mg/kg)

Investigations of Enzymes Linking Oxidative Stress

Rinsing the mammary tissue with a normal saline solution that was extremely cold was followed by rinsing the tissue with 0.15 M From that point onward, the acidity of Tris hydrochloric acid (pH 7.4) following procedures were carried out.

(a) Lipid Peroxidase (LPO) -TBARS are produced when TBA combines with MDA in an acidic media (pink color)**.** In a buffer containing 0.15 M tris HCl, prepare a tissue homogenate that is 10% weight/volume (pH 7.4). 0.2 milliliters of tissue homogenate combined with 0.2 milliliters of 8.1% SDS, 1.5 milliliters of 20% acetic acid, and 1.5 milliliters of 8% TBA (makeup volume up to 4 ml with distilled water). Heat in a water bath at 95 degrees Celsius for sixty minutes while using a glass ball as a condenser. After it has cooled, bring the volume back up to 5 ml. Add 5 ml of butyl: pyridine (15:1). Vortex 2 min. Centrifugal at 3000 rpm (10 min). Take the optical density reading at 532 nm of the top mammary tissue layer [(blank – butanol: pyridine / 15:1)], (this absorbance will be the total MDA that has been generated]. The standard calibration curve of MDA will be used as a basis for interpretation[12][13].

(b) Superoxide Dismutase (SOD) -To experiment, superoxide is generated from oxygen by employing decreased b-nicotinamide adenine dinucleotide (NADH) as just a reduction agent and phenazine methosulphate (PMS) as a catalyst. When this is done, it is done in the presence of an indicator known as nitro blue tetrazolium (NBT), which becomes blue when it is reduced by superoxide. Spectrophotometry allows for the tracking of color changes in the visible spectrum, namely at 560 nanometres. When normal amounts of beverages are introduced to the process, the antioxidants in the beverages interact with NBT to deal with the superoxide. This causes the reaction to proceed more slowly. The degree to which one can arrest the decrease of NBT can be used as a measurement for superoxide scavenging [12][13].

c) Glutathione, also known as GSH- Create a homogenate of the tissue using 0.1 M phosphate buffer with a pH of 7.4 and 10%. Get 0.2 milliliters of the homogenate. Include 20% TCA and 1 mM EDTA in the mixture. Set aside for 5 min. Centrifuge 10 min at 2000 rpm. Take the supernatant, which should be about 200 l, and transfer it to a new tube. Add 1.8 milliliters of Ellman's reagent, which is 5,5'-dithiol bis-2-nitrobenzoic acid (0.1 millimolar) produced in 0.3 millimeters of phosphate buffer, pH 7, with 1% sodium citrate solution. Distilled water should be used to get the volume up to 2 milliliters. Take OD at 412 nm (water as blank) [12][13].

d) Catalase -2.9 ml of H2O2 solution was taken and A240 nm was observed. Added 0.1

ml test sample when the A240 was stable and the time required for the A240 nm to decrease from 0.45 to 0.40 absorbance units was noted (Goth, 1991; Sapakal, 2008). Unit/ml enzyme = (3.45 x df) / (min x 0.1) Corresponds to the decomposition of 3.45 micromoles of hydrogen peroxide in a 3.0 ml reaction mixture producing a decrease in the A240nm from 0.45 to 0.40 absorbance units Where- df: Dilution factor (final volume/test sample volume) Min: Time in minutes required for the A240nm to decrease from 0.45 to 0.40 absorbance units 0.1 Volume (in milliliter) of enzyme used Unit/mg tissue = (Units mg/ml) / (mg tissue/ml enzyme)[12][13].

The Definition of Units -One unit will break down 1.0 micromole of hydrogen peroxide every minute at a temperature of 25 degrees Celsius and a pH of 7.0. At the same time, the concentration of hydrogen peroxide will drop from 10.3 mM to 9.2 mM. One can monitor the rate at which H2O2 is being removed from the system by keeping an eye on the rate at which the absorbance at 240 nm is decreasing as time passes[12][13].

StatisticalAnalysis - Usingstat3.2software,Datawereexpressedasmean±StandardDeviation(S.D).Allof the data were compared using one-way ANOVA with Dunnett's post-test. If ≤ 0.05, a variance was considered statistically significant.

3.RESULT

Phytochemical examination

Tannins (using FeCl3 test, K2Cr3O7 test, and lead acetate test), glycosides (using Legal's test, Kellertest, Killiani's, and Borntrager's test), and flavonoids (using Legal's test, Keller-test, Killiani's and Borntrager's test) were tested as phytochemical groups. vitamins (as determined by Legal's test, Keller's test, Killiani's test, and Borntrager's test). Saponins (as determined by the foam test), alkaloids (as determined by Mayer's test, Dragendorff's test, Wagner's test, and Wagner's test). Sugar reduction (using Fehling's and Benedict's tests), flavonoid reduction (using Shinoda and Benedict's tests), zinc hydrochloride reduction test, phytosterols (using Liebermann-test Burchard's and the Salkowski reaction), proteins and amino acids (using the biuret and ninhydrin tests). polyphenols (as evaluated) (FeCl3 test). Polyphenol saponins, glycosides, triterpenes, tannins, and flavonoids were founded in *Russelia equisetiformis*hydroalcoholic extract.

CharacterizationofHERE-AgNPs

The morphological study was performed by SEM and obtained photomicrographs are presented in Figures 3a and 3b. SEM images of formulated silver nanoparticles of RE plant extract are shown at different magnifications. It can be seen that core particles were of spherical shapes (Fig.1a) with size variation from 40.35 to 90.47 nm average particle size was found to be around 80.51 nm moreover few cubic and road-like nanostructures were also found at 10x magnification (Fig. 1b) using an advanced software named “IMAGEJ’. The big size variation could be due to the agglomeration of individual nanoparticles or subunits into larger particles. The EDX data shows energy dispersion. Fig4 HERE-AgNPs are surface bio-fabricated nanoparticles consisting of C, Ago, Cl, and O. The silver is reported to be 60% with 35% of carbon, confirming that the nanoparticles are free of contaminants.

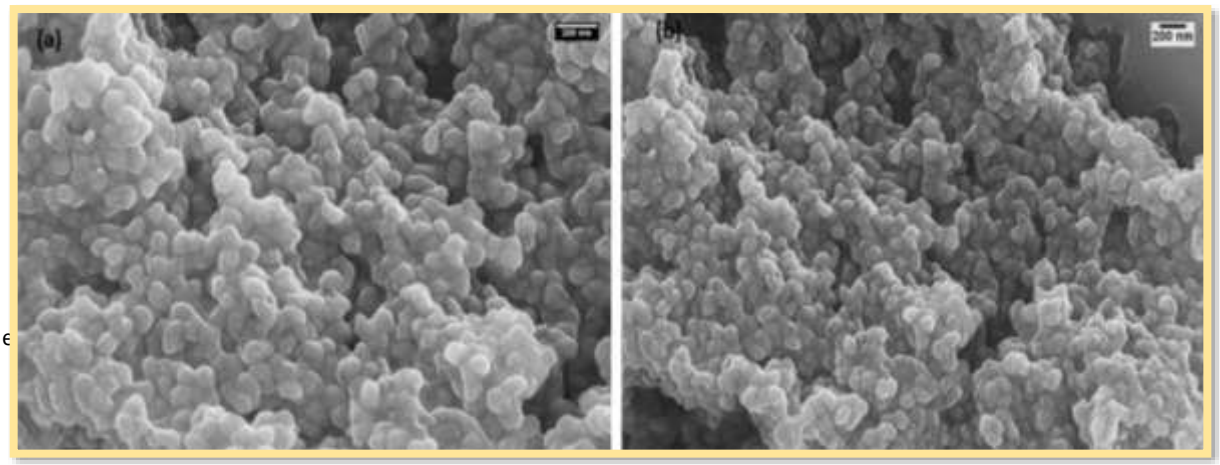

*Fig-01(a&b) SEM*of HERE-AgNPs

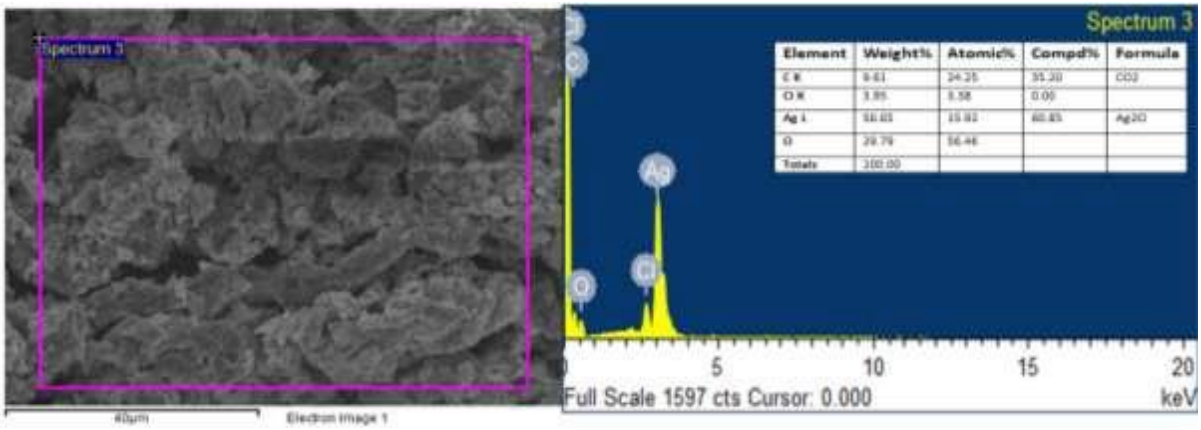

*Fig-02 EDX*ofHERE-AgNPs

Acute toxicity studies

All the animals gained weight and showed no signs of behavioral alteration, indicating that the administration of both (HERE) and HERE AgNPs) extracts had a minor effect on the animals' growth. LD50 values were shown to have concentrations of more than 2,000 mg/kg across all of the dosages tested and whole toxicity studies found that HEREare no deaths or clinical symptoms of toxicity at any of the doses examined (Table-6.5). When given to rats at a dosage of 2000 mg/kg, it has been shown that both the extract HERE and HERE-AgNPs formulations are not deadly. As a result, a 1/10th dose of 200 mg/kg was chosen as the high dose for both HERE and HERE AgNPs, and a 100 mg/kg dose is being evaluated for additional biological investigations. The results of the observations are listed in the following table:

Table -02: Mortality at various doses in Acute oral toxicity studies					
S.no	Test Sample (mg/kg)	05	50	300	2000
1	HERE	None	None	None	None
2	HERE-AgNPs	None	None	None	None

***In vivo* anti-inflammatory activity:**

Pro-inflammatory cytokines IL-6 and anti-inflammatory cytokine IL-10 have centralroles in the process of inflammation. IL-6 contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions.IL–6 levels changed anddecreases the level whereas IL-10 As shown in Figure 2 comparatively increased but when compared to the negative control group its was continuing significantly decreased

Table -03			
S.no	**Group**	**IL-6 (ng/ml)**	**IL-10 (pg/ml)**
1	G-1 (Controls Group)	1.88±0.51	3.85±0.25
2	G2(Negative control- DMBA)	9.66±.058	8.58±0.30
3	G3(Only Extract HERE-100mg)	3.51±0.55	5.01±0.25

4	G4 (HERE-AgNPs-100mg)	3.95±0.56	5.21±0.22
5	G5 (HERE -AgNPs-200mg)	3.95±0.49 [a]	4.02±0.21 [a]
6	G6 (Tamoxifen -Standard)	2.27±2.30[a]	3.21±0.14 [a]
Value is expressed as mean ±SD for all six animals in four groups Superscript "a" is donated as the significant (P <0.001) and b is (P< 0.002)			

Biochemical Assays for evaluation of oxidative stress

The levels of oxidative stress markers in rat mammary tissue homogenate were measured. These markers included lipid peroxidation (TBARS), SOD, catalase, and glutathione in the brain tissue of experimental rats, the amounts of antioxidant enzymes such as Superoxide, CAT, and GSH were considerably (P 0.01) enhanced when compared to the levels in control rats. by HERE 100 mg, HERE-AgNPs 100 and 200mg/kg, as shown in Figure-6.12 (a-d). Additionally, the level of antioxidant activity was dose-dependently increased from G3 to G5. In contrast hand, administration of HERE at a dose of 100 mg/kg caused changes in the concentrations of enzymatic antioxidants in the breast tissue of less significant rats.

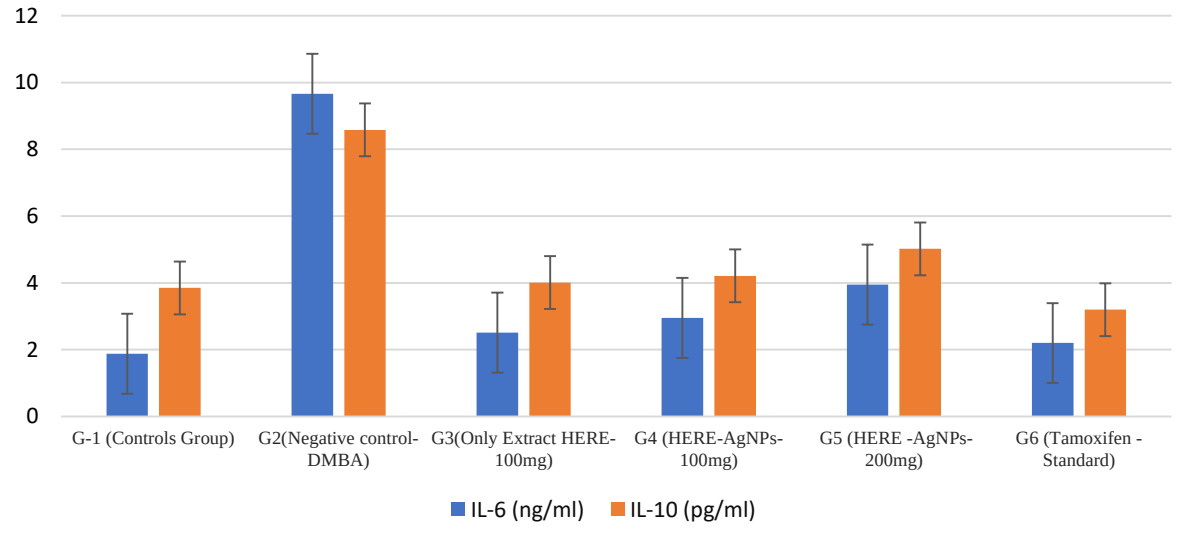

Fig.03 IL-6 and IL-10 (Anti-Inflammatory Activity)

Table 04: Biochemical Assay to the evaluation of oxidative stress

Group	LPO (nM MDA/gm moist tissue)	SOD (U/gm moist tissue)	GSH measured in nmol per gram of moist tissue	catalase (Units per gram of moist tissue)
	Mean ±SD	Mean± SD	Mean± SD	Mean± SD
G-1 (Control Group)	36.43±3.37	39.07±4.67	1.032±0.19	4.425±0.49
G2(Negative control- DMBA)	98.24±3.93	12.85±2.06	0.25±0.05	1.24±0.13

G3(Only Extract HERE-100mg)	78.78±6.95[a]	23.72±4.33[b]	0.56±0.21[b]	1.93±0.39[c]
G4 (HERE-AgNPs-100mg)	71.59±3.09[a]	30.26±3.768[a]	0.64±0.091[a]	2.34±0.314[a]
G5 (HERE -AgNPs-200mg)	66.66±5.29[a]	28.62±4.05[a]	0.79±0.05[a]	2.35±0.25[a]
G6 (Tamoxifen - Standard)	44.72±4.85[a]	37.11±3.88[a]	0.97±0.14[a]	2.51±0.58[a]
For all six animals in four groups, the value is presented as the mean ±SD. The significance (P <0.001) is supplied by superscript "b," and c is (P <0.002).				

1260

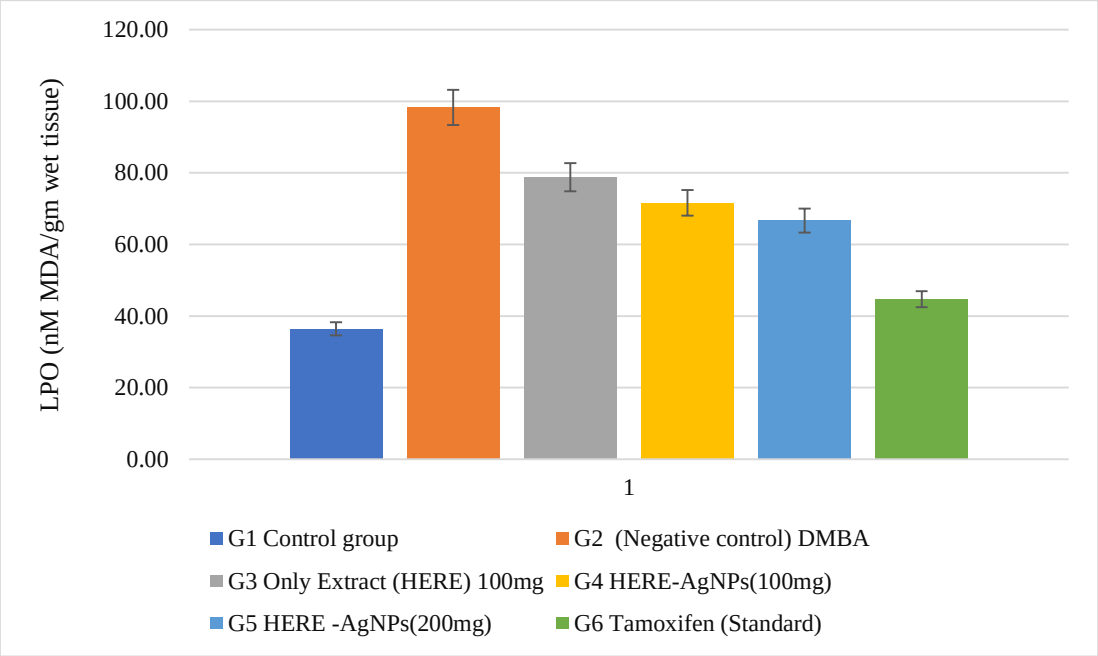

Figure -4(a)LPO

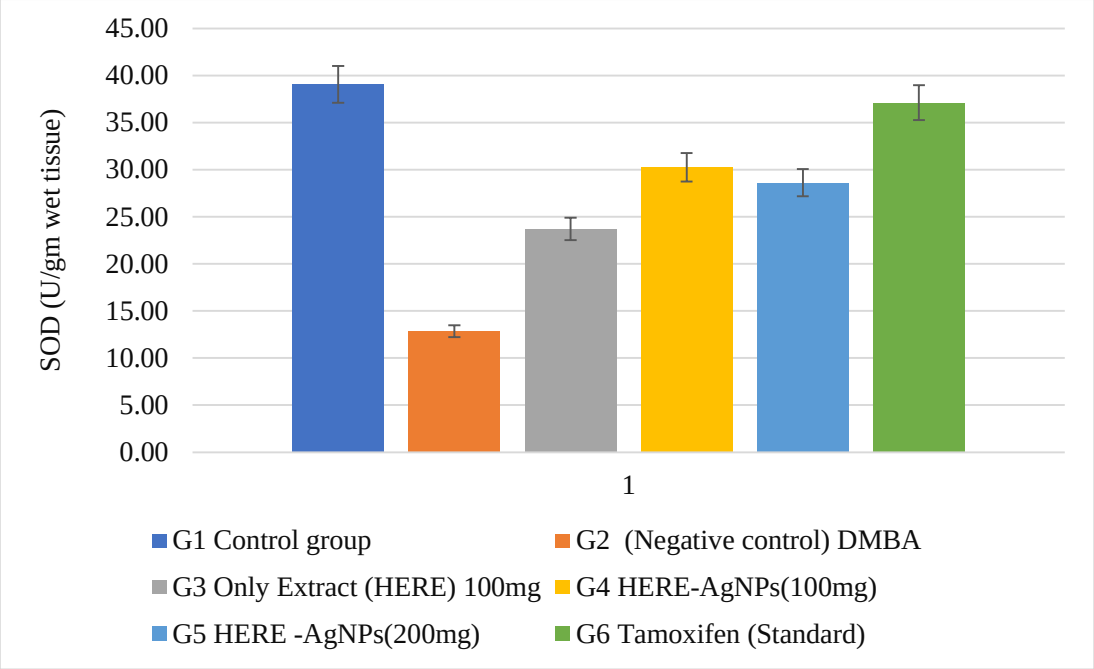

Figure -4 (b) SOD

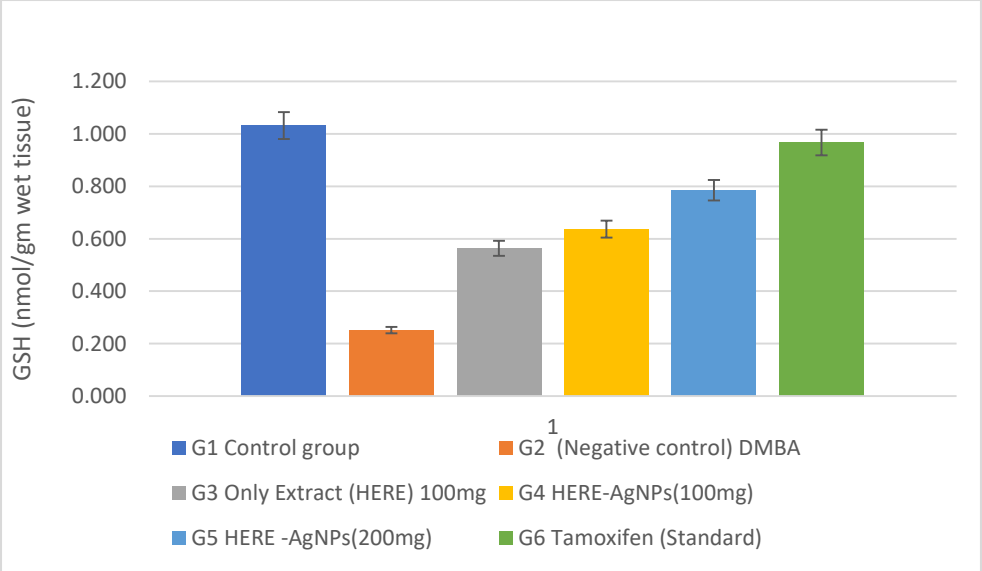

Figure-4 (c)GSH

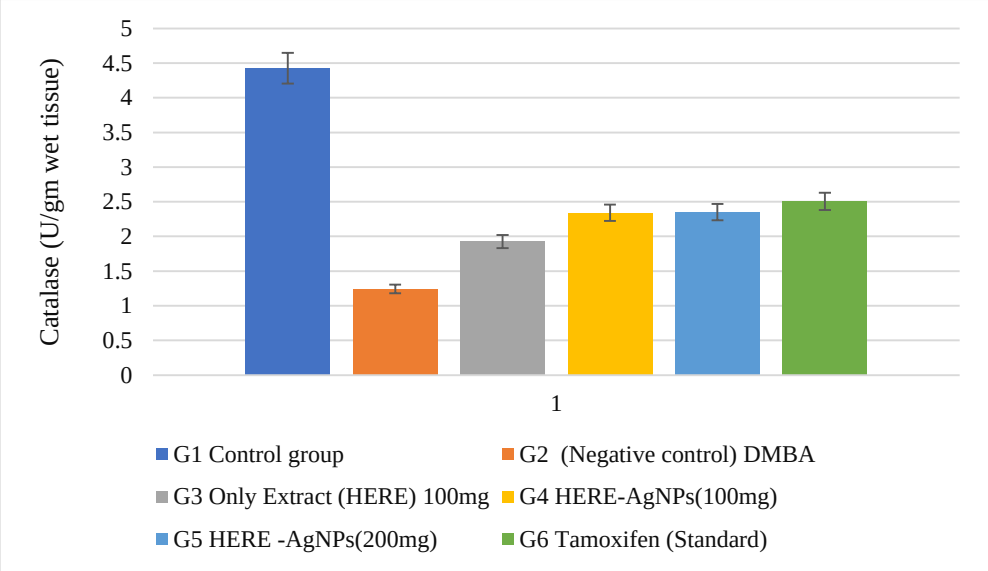

Figure-*4* (d) Catalase

4. DISCUSSION

Synthesis of nanoparticles has developed rapidly in recent years compared to the beginning of the century. Previously, physicochemical methods were involved in nanoparticle synthesis. It takes less time to synthesize nanoparticles in large quantities using conventional physical and chemical methods but requires toxic capping chemicals to maintain stability, resulting in environmental toxicity. increase. With this in mind, plant-based green nanotechnology has emerged as an eco-friendly alternative, as biosynthesis of nanoparticles via plant extracts is inexpensive. Therefore, this experiment was conducted to evaluate the anti-inflammatory and antioxidant properties of silver nanoparticles from *Russelia equisetiformis* flower extract. The current study found polyphenol saponins, glycosides, triterpenes, tannins, and flavonoids in the hydroalcoholic extract of *Russelia equisetiformis*. For these reasons, *Russelia equisetiformis* was employed as a natural reducing agent in the green production of silver-based nanoparticles. The outcome of acute toxicity reveals that there is no toxicity found in both *Russelia equisetiformis*Hydroalcoholic extract (HERE) and silver nanoparticles of *Russelia equisetiformis*Hydroalcoholic extract (AgNPs-HERE). Oxidative stress biomarkers Oxygen is indispensable for all the aerobicspecies but the reactive forms of oxygen such as superoxide (oxygen with an extra electron) can lead to certain disasters. To combat this phenomenon, the organism has several mechanisms which keep these reactive species in control as they are also important in several beneficial processes. However, several processes are interdependent because they share many metabolites and products. The antioxidant enzyme can be induced under slight oxidative stress but severe oxidative stress can lead to the suppression of these enzymes. Lipid peroxidation is a complex process that takes place in aerobic cells and reflects the connection between oxygen molecules and polyunsaturated fatty acids. This process may be thought of as a chain reaction. The oxidation of lipids is the name given to this process. The free - radicals are liable for peroxidation by lipids, which is also due to the decomposition of foods, the speed of the process of aging in animals, and the development of cancer. The oxidation of macromolecules (such as proteins, amino acids, lipids, and DNA) is caused by these free radicals as well as other related species. This process eventually leads to damage to the cell as well as the death of the cell. Lipid peroxidation has been recognized for some time as a toxicological process that, when it occurs in biological systems, is capable of

producing a wide range of unfavourable results. This misconception is still widely held today. The process of lipid peroxidation results in the formation of reactive aldehydes. The aldehydes malondialdehyde and 4-hydroxyl nominal are both good examples of reactive aldehydes. Numerous examples of these reactive aldehydes may cause severe damage to cellular structures. In addition, MDA can cause disorders that are neurological, cytotoxic, and cancerous when it combines with other biomolecules. On the other hand, endogenous antioxidants such as catalase and superoxide dismutase are susceptible to the oxidative changes that might take place in the body. The enzyme SOD oversees properly disposing of the superoxide anion, and the CAT protein, which is a protein, oversees catalytic the reductions of H2O2 and guarding the tissues against the very hazardous hydroxyl radicals that may be created by H2O2. GSH acts as the body's first line of defence by eliminating reactive oxygen species. This is how it fulfils its function (ROS). Oxidative stress leads to a decrease in glutathione levels by inhibiting glutamate-cystine antiporter and causes glutathione to be oxidized to glutathione disulfide in a fast, non-enzymatic manner. These electrophilic molecules include free radicals and reactive oxygen species (ROS).IL-6 and IL -10 levels were significantly decreased as compared to the DMBA-induced group moreover *Russelia equisetiformis* Hydroalcoholic extract also significantly downthe level of IL-6 and IL -10 level.

5. CONCLUSION

The conclusions of the current study According to this study, *Russelia equisetiformis* has no direct activity on cancer cells, but it functions mostly through decreasing oxidative stress, slowing cell growth, and causing cells to die by apoptosis in DMBA-induced mammary tumour cells and other types of cancer cells. Carcinoma. To the best of our knowledge, this is the first publication on the synthesis and characterisation of AgNPs from A. aqueous *Russelia equisetiformis* flower extract, as well as research into their antioxidant, anti-inflammatory, and anti-cancer effects. The nanoparticles created were random, spherical in shape, and crystalline in nature. The optimised biosynthesized nanoparticles demonstrated excellent anti-inflammatory and antioxidant activity as well as therapeutic potential**.**

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