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Green-synthesized Copper Ferrite Nanoparticles by using *Glycyrrhiza glabra* bark extract with Anti-oxidant, Anti-inflammatory, Antidiabetic, Antiurolithiatic, Anticoagulant, Thrombolytic and λ-DNA damage protection properties

Priyanka P.S[a], Ashwini C A[a], Shivu C[a], Lokesh R N[a], Sujatha M Hanumegowda[a], Manjunatha H[a], Vijaykumar Manjunatha[b] Chandramma Srinivasa [a*], Prathiba H.D [a*]

[a] *Department of Biochemistry Bangalore University Jnanabharathi Campus Bangalore- 560056*
[b] *Harsha institute of allied health sciences, Bangalore-562123.*

ABSTRACT

The safety, efficacy, and utility of medicinal plants have become an issue of concern due to their increased use. Therefore, the importance of a comprehensive knowledge of the phytochemical compounds of medicinal plants is essential since it will be instrumental in the development of new medicinal compounds. The various uses of copper nanoparticles (CuNPs) have become an important topic of interest. The aims and objectives of the research were to use the bark extract of *Glycyrrhiza glabra* to develop and characterize copper ferrite nanoparticles (CuFe2O4 NPs). The reaction between iron nitrate $Fe(NO_3)_3.9H2O$ and copper nitrate $Cu(NO_3)_2.3H_2O$ and the bark extract of *Glycyrrhiza glabra* produced CuFe2O4 NPs. The presence of copper oxide NPs was confirmed using UV-Vis spectroscopy. The UV-Vis spectroscopy was instrumental in confirming the presence of the NPs since it showed absorbance between 350 and 480nm. The safety, efficacy, and utility of medicinal plants have become an issue of concern. As a result, the importance of a comprehensive knowledge of the phytochemical compounds of medicinal plants is essential since it will be instrumental in the development of new medicinal compounds. Copper nanoparticles (CuNPs) have received a lot of interest due to their various uses. The objectives of this study were to green synthesise and characterise Copper Ferrite Nanoparticles ($CuFe_2O_4$ NPs) by using *Glycyrrhiza glabra* bark extract. The $CuFe_2O_4$ NPs was prepared by the reaction between Iron nitrate $Fe(NO_3)_3.9H_2O$, copper nitrate $Cu(NO_3)2.3H_2O$ with Glycyrrhiza glabra bark extract . Based on UV-vis, FTIR, EDEX, Raman spectroscopy as well a XRD analysis. Uv-Vis spectrometry determined the absorbance from 350 to 480nm indicated that the presence of copper oxide nanoparticles. In FITR major peaks observed at 2912 , 1992, 1968, 1557, 1011 and 418 cm−1 with functional groups of Methylene(CH2) Methylene C-H asymmetry /sym. stretch, Transition metal carbonyls, Transition metal carbonyls, Alkenyl C=C stretch Aryl-substituted C=C Conjugated C =C, Cyclohexane ring vibrations and Polysulfides (S-S stretch) respectively. Three major peaks with 2 θ were found to be 46.240 0, 62.1080 and 78.4080 with respect to β of 0.42, 0.209 and 0.214 respectively and 22.59nm crystallite size, D, was calculated. The morphology of $CuFe_2O_4$ NPs was exists in crystalline-like structure confirmed by SEM. PXRD analysis. EDAX was confirmed that the mostly CuFe2O4 NPs with the existence of Cu, Fe and trace amount of Si. CuFe2O4 NPs shows the sharp peak at 709 cm−1 along with the smaller peak at 236 cm−1 in Raman spectrometry. The antioxidant activity of GG.CuFe2O4 NPs was confirmed by Hydrogen peroxide radical scavenging and reducing power assay. The 50µg of GG.$CuFe_2O_4$ NPs scavenges 80.00% and 53.33 of H_2O_2 radicals and FRAP value respectively compared with standard Ascorbic acid (80.63% and 77.66% respectively). GG.$CuFe_2O_4$ NPs exhibited antihemolytic property confirmed the non-toxic effect at the dose of 400 µg. The GG.$CuFe_2O_4$ NPs showed the inhibition of egg denaturation and BSA denaturation percentage about 80.63% compared with acetylsalicylic acid positive control (85.00%). Human RBC membrane against heat induced lysis was inhibited by GG.$CuFe_2O_4$ NPs with 53.40% and Acetylsalicylic acid standard drug showed 61.54% Inhibition. GG.$CuFe_2O_4$ NPs (100 and 500µg) exhibited fair anti-inflammatory property that was tested using egg albumin and BSA denaturation denaturation percentage about 68.73% and 78.63% compared with acetylsalicylic acid positive control (74.66.%and 83.23% %) respectively. Prevention of λ-DNA damage by GG.$CuFe_2O_4$ NPs confirmed the DNA protection at 50 µg of dose. The effects of GG.$CuFe_2O_4$ NPs s(30µg) on glucose uptake in yeast cells upto 61% confirmed the antidiabetic activity. Antiurolithiatic activity of GG.$CuFe_2O_4$ NPs was depicted by percent inhibition of nucleation 58.33%. The GG.$CuFe_2O_4$ NPs showcased anticoagulant effect as increased clotting time of PRP from control 306 to 2400s which inhibited blood coagulation. Thrombolytic activity of GG.$CuFe_2O_4$ NPs dissolved the blood clot with 86% at the concentration of 600µg and the streptokinase (60U/ml) was taken as positive control with 66.8%. of clot lysis percentage.

Keywords: *Glycyrrhiza glabra*, Heamolysis, Anti-oxidant , Anti-inflammatory Antiurolithiatic, Anticoagulant and Thrombolytic and λ-DNA damage.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



INTRODUCTION

Nanoparticles are incredibly tiny, with sizes ranging from 1 to 100 nanometers. They are utilized in many different fields, such as electronics, optics, agriculture, and medicine, due to their small size and great versatility [1]. They aid in the creation of more compact and effective electronics. In optics, they improve the quality and performance of materials that interact with light [2]. They can improve the delivery of pesticides or fertilizers in agriculture. They aid in medical imaging and targeted drug delivery, which enhances the precision and effectiveness of treatments [3]. In the quickly expanding subject of bionanotechnology, bioorganisms are often employed to create nanomaterials, which are then used to enhance the organisms' quality of life [4]. Microbial enzymes or plant phytochemicals employ the biological principle of oxidation and reduction in biological synthesis [5]. These days, the synthesis of inorganic NPs is mostly accomplished by physical and chemical means. There are drawbacks to both physical and chemical processes, including low productivity, toxicity, non-eco-friendliness, and high capital requirements [6]. Because of these factors, biological synthesis is attempting to take the place of chemical NP production techniques. This green synthesis method has already produced many metal/metal oxide nanoparticles, including silver (Ag), gold (Au), selenium (Se), platinum (Pt), zinc oxide (ZnO), iron oxide (Fe2O3), and graphene oxide, according to numerous papers and research [7]. CuNPs have lately drawn attention, although other studies have shown that a number of metal nanoparticles have a range of biological and biochemical properties [8]. In humans, copper serves as a cofactor for many enzymes involved in the synthesis of neuropeptides, the control of cell signaling pathways, antioxidant defense, and immune cell activity [9]. Plants require copper for a number of physicochemical and metabolic functions. As cofactors for several enzymes, it aids in the regulation of various metabolic and biological processes [10]. As a cofactor for several enzymes, it is in charge of the proper operation of various important proteins and enzymes, including amino oxidase, cytochrome c oxidase, and plastocyanin [11]. Conversely, the antimicrobial, antibacterial, antifungal, magnetic phase change, gas sensing, biocidal, superconductive, catalytic, and optical capabilities of copper oxide have been reported [12]. In this regard, the use of biologically produced copper nanoparticles (CuNPs) was discovered to be a promising bioactive agent. It can be used for various biological and medicinal purposes [13]. Due to the widespread belief that plant-based substances are safer and more health-promoting, there has been an increase in consumer interest in natural medications in recent years [14]. Additionally, sectors including food, cosmetics, nutraceuticals, and pharmaceuticals are searching for plants that have historically been used in medicine to incorporate into their products as a result of this trend [15]. Licorice, or *Glycyrrhiza glabra Linn*., is one such plant that has been utilized for ages because of its therapeutic qualities [16]. It is a member of the Fabaceae family and the Glycyrrhiza genus, which has more than 30 species and is found all over the world. It has been used for centuries in many cultures,

including Ancient Egypt, Rome, Greece, East and Chin [17]. Glycyrrhizin, 18β-glycyrrhetinic acid, glabrin A and B, and isoflavones are among the many bioactive substances found in licorice. Pharmacological research has shown that these phytochemicals are thought to contribute to a variety of health advantages [18]. Antibacterial, anti-inflammatory, antiviral, antioxidant, immune-stimulating, and antidiabetic properties have been demonstrated by *Glycyrrhiza glabra* preparations and specific components [19]. Licorice has demonstrated potential in the treatment of long-term illnesses such cancer, heart problems, respiratory disorders, and liver protection [20].

The aim of the study was to assess the anti-haemolytic, antioxidant and anti-inflammatory properties of copper nanoparticles green synthesized by using *Glycyrrhiza glabra* bark extract has been explored. Green synthesized Copper ferrite NPs were eco-friendly and considered a promising candidate for use in nanomedicine, a field dedicated to improving human health through nanotechnology. Nanomedicine leverages the unique properties of nanomaterials to enhance drug delivery, diagnosis, and therapeutic effectiveness.

MATERIALS AND METHODS

Calcium chloride, hydrogen peroxide, fresh human blood was collected from healthy donors), Bovine serum albumin (BSA), Phosphate buffer, EDTA (ethylene diamine tetra acetic acid). acetyl salicylic acid (aspirin), Iron nitrate $Fe(NO_3)_3.9H_2O$, copper nitrate $Cu(NO_3)_2.3H_2O$, were incurred from Sigma Aldrich company, St. Louis, USA. All the chemicals are analytical grade.

Preparation of *Glycirrhiza glabra* bark extract and Synthesis of Copper Ferrite Nanoparticles ($CuFe_2O_4$ NPs)

Glycyrrhiza glabra bark was bought from the store and dried in hot air oven for 1 hour, the dried bark was macerated with the help of pestle and mortar to obtain a fine powder. then 2.4 g of fine powder was weighed and added to 200 ml of distilled water stirred the mixture for 1 hour at 70^0C and filter the extract then add 0.944g cupric nitrate and 3.23g of iron nitrate to the filter the supernatant. Then stir the mixture at 800^0C for 45min, while stirring add a few drops of ammonia (salt) to get pH-9.0. Then precipitate and heated at 200^0C to get a coloured powder, and calcinated the powder at 600^0C for 4 hours. The obtained filtrate was dissolved in water and it has been named as *Glycyrrhiza glabra* Copper Ferrite nanoparticle ($GG.CuFe_2O_4$ NPs) used for further assay.

Characterization of $GG.CuFe_2O_4$ NPs

The final product of brown color powder that was carefully collected and stored for characterization. To get a finer nature for characterization and analyze the produced $GG.CuFe_2O_4$ NPs like Ultra Violet-vis spectroscopy (UV–Vis), powder X-ray diffraction (PXRD), a technique used to analyze the structure and phase confirmation of nanoparticles. Fourier transform infrared spectroscopy (FTIR) was used to analyze the functional group present in the nano-composite. Scanning electron microscopy (SEM) was used to study the surface morphology and particle distribution. Elemental analysis of nanoparticles was done by energy dispersive X-ray analysis (EDAX). Raman spectroscopy was used to determine the arrangement no atoms and bonds with in a molecules.

Evaluation of Anti-oxidant Activity

Hydrogen peroxide radical scavenging activity

The assessment of hydrogen peroxide scavenging activity (HPSA) was conducted by the keshari [21] method with minor adjustments. Specifically, $GG.CuFe_2O_4$ NPs (concentrations ranging from 50µg/ml to 250µg/ml) were combined with 3.4 mL of 0.1M phosphate buffer (pH 7.0) and 0.5ml of a 40mM H_2O_2 solution. The solution was subjected to a 10min incubation period, during which absorbance measurements were taken at a wavelength of 230 nm against a blank containing only phosphate buffer. As control phosphate buffer with H_2O_2 is employed. Subsequently, absorbance readings at 230 nm were obtained, and the findings were reported as in percentage. Then percentage of hydrogen peroxide scavenging activity was calculated as: HPSA (%) = (OD) (control) OD (sample))/ (OD (control))×100 formula no 4. OD (control) = optical density of control, OD (sample) = optical density of sample.

Ferric reducing antioxidant power assay

The reductive potential of the GG. $CuFe_2O_4$ NPs was determined using the Yen and Chen technique [22]. Different concentrations of GG. $CuFe_2O_4$ NPs (10–50 µg) in 0.5 mL of water was mixed with equal volumes of 0.2 M phosphate buffer, pH 6.6 and potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated the test tubes in water bath for 20 min at 50°C. At the end of incubation, an equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 ×g for 10 min. The supernatant was mixed with distilled water and 0.1% ferric chloride at 1:1:0.2 (v/v/v), and the absorbance were measured at 700 nm. An increase in the absorbance of the reaction mixture indicates the potential reducing power of the sample. AA was used as a standard for comparison.

Percentage of FRAP was calculated using the following formula:

% of reducing activity = $A0 - A1/ A0 \times 100$

where A0 was the absorbance of the control solution and A1 was the absorbance of the reaction mixture.

Preparation of 10% Haematocrit

RBCs were collected from healthy human individuals aged from 23 to 35 and mixed with trisodium citrate at the ratio of 9:1 (v/v) then span at 800-900rpm for 13min. The platelet-rich plasma (PRP) was collected at supernatant, and RBCs were collected as a pellet. The collected ghost RBCs were washed thrice with 10mm/L PBS (pH 7.4). From the ghost RBCs, 10% haematocrit was prepared by using 10 mm/L PBS, and the same was used for the further assays.

Direct Haemolytic Activity

The direct hemolytic assay was carried out as described by Stoykova et al. [23]. Packed erythrocytes from human and phosphate buffer saline were taken in the ratio of 1:9. Suspension of hematocrit (1ml) was incubated with various concentrations of GG.$CuFe_2O_4$ NPs (100-400µg) independently for 1h at 37°C. The reaction was terminated by adding 9ml of ice-cold PBS and centrifuging at 1000g for 10min at 37°C. The amount of hemoglobin released in the supernatant was measured at 540nm. The percentage of hemolysis was expressed against 100% lysis by water (positive control) and PBS served as a negative control.

In vitro Anti-inflammatory Activity

Membrane Stabilization by Heat-Induced Hemolysis

The experiment was performed according to Gonfa, Y.H et al. [24]. The reaction mixture was incubated with various concentrations of GG.$CuFe_2O_4$ NPs (10-50µg) along with 1ml RBCs suspensions. For control instead of test sample only saline was added. Aspirin was taken as a standard drug. The tubes were further incubated in water bath at 56°C for 30min. Incubated tubes were cooled and the reaction mixture was centrifuged at 2500rpm for 5min and the absorbance was recorded at 560nm. The absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula.

% induced haemolytic = (OD (control) -OD (sample))/ (OD (control)×100 formula

Egg denaturation assay

The method of Paul, S [25] was followed for the inhibitory assay of egg albumin denaturation. Briefly, 3 mL of reaction mixture consisting of 0.2ml of egg albumin and 2.8ml of 0.1M phosphate buffer saline (pH 6.4) was added to different concentrations of GG.$CuFe_2O_4$ NPs (20–400µg). Aspirin (100 µg) was taken as a standard drug control. The reaction mixtures were incubated at 37^0C for 15min and then incubated at 60 ^{0}C for 10min in a water bath. The samples were cooled at room temperature, and the turbidity of the sample was measured at 660nm. The percentage inhibition of egg albumin denaturation was calculated by using the formula.

(%) of inhibition = 1- (OD(control) -OD (sample))/ (OD (control)×100

Inhibition of BSA Denaturation

The method of Gunathilake, K et al. [26] was employed to carry out the inhibition of BSA denaturation. Briefly, 1.0 ml of 1% bovine serum albumin was pre-incubated with GG.$CuFe_2O_4$ NPs (100-500 µg) . By adding 1.0ml of 0.1M phosphate buffer saline (pH 6.4). The reaction mixture was incubated at 37^0C for 15mins in incubator. Thereafter, the reaction mixture was heated at 60°C for 5min in a water bath. After cooling of reaction mixture, the turbidity was measured at 660nm by using UV–VIS spectrophotometer. The percentage inhibition of BSA denaturation was calculated by using the given below formula.

Percentage inhibition of denaturation = 1 − OD of sample/ OD of control × 100

Prevention of λ DNA damage by GG.$CuFe_2O_4$ NPs

Oxidative λ-DNA damage was prevented by GG.$CuFe_2O_4$ NPs and it was assayed [27] . λ-DNA (0.5µg) with and without GG.$CuFe_2O_4$ NPs (50µg) was Subjected to UV irradiation for 1hr. Relative difference between oxidized and native DNA were analyzed on 1% agarose gel prepared in tris-acetate-EDTA buffer (pH 8.5) at 50V for 3hrs at room temperature. The gel was documented (GEL DOC) and the band intensity was determined

Invitro Antidiabetic Activity of GG.$CuFe_2O_4$ NPs

In order to carry out this experiment according to Shettar AK et al. [28]. Commercially available yeast was washed by repeated centrifugation at 3,000rpm for 5min in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. was added to different concentrations of GG.$CuFe_2O_4$ NPs (5–30µg) and incubated for 10min at 37 °C. Reaction was started by adding 0.1 ml of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500rpm, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard drug (500µg). The percentage increase in glucose uptake by yeast cells was calculated by using following formula.

(%) of glucose uptake = (OD (control) -OD (sample))/ (OD (control)×100

Antiurolithiatic assay

The effects of crystallization was investigated by Bashir S method [29]. 5Mm calcium chloride and 7.5mM sodium oxalate solutions were prepared in a buffer (Tris-HCl, 0.05M + NaCl, 0.15M) having pH 6.5. Various dilutions of cystone (100µg) were prepared in distilled water. 40µl of each dilution Cystone, added to different concentrations of GG.$CuFe_2O_4$ NPs (5–25 µg), were mixed with 2.5mg calcium chloride and then sodium oxalate 2.5mg was added in each dilution These dilutions were incubated in oven (37°C) for 30 minutes. Then, after cooling the optical density was noted at 620nm.

Percent inhibition of Anti Urolithiatic assay was calculated from the following formula:

$$\text{Percent Inhibition} = 1 - \frac{\text{OD of Sample}}{\text{OD of Control}} \times 100$$

Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP)

PRP and PPP were prepared based on the method described by Ardlie and Han [30]. The platelet concentration of PRP was adjusted to 3.1×108 platelets/ml with PPP. The PRP maintained at 37°C was used within 2hr. PRP was prepared using plastic wares or siliconized glass wares.

Plasma re-calcification time

The plasma re-calcification time was assayed based on the method described by Quick et al., [31]. GG.$CuFe_2O_4$ NPs (1000-1200µg) was pre-incubated with 0.2ml of citrated human plasma in the presence of 10mM Tris HCl (20µl) buffer pH 7.4 for 1min at 37°C.

Blood clot lysis study

whole blood clot lysis was done according to the method of Prasad et al. [32]. whole blood was taken into sterile micro centrifuge tubes (500µl/tube) and incubated at 37°C for 45min to form the clot. Later, the weight of the clot was measured, and different concentrations of GG.$CuFe_2O_4$ NPs (200-600µg) were added to all the tubes and incubated at 37°C for 90min, then observed for clot lysis. Discarded the

serum and weighed it again in order to record the variance in weight after clot disruption. The blood clot lysis was expressed in percentage. The data are presented as mean ± SD (n=3).

Statistical Analysis

Results are articulated as the mean value of three independent experiments ± standard deviations. Statistical difference between the compounds was tested by one-way analysis of variance (ANOVA) using the GraphPad Prism 5, and individual comparisons were obtained by Dunnett's multiple comparison test. The probability value (p) less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Synthesis and Characterization of GG.$CuFe_2O_4$ NPs

The GG.$CuFe_2O_4$ nanoparticles have been studied using UV-Vis spectroscopy (Shimadzu) to investigate the optical absorbance characteristics of the nanoparticles. Figure 1 illustrates the results of the analysis, in which the nanoparticles show an absorbance range of 350-480 nm. These results verify the successful synthesis of GG.$CuFe_2O_4$ nanoparticles. The optical absorption of CuO nanoparticles was studied using a UV-Visible spectrophotometer over a range of 200-800nm. A similar absorption peak in the nanoparticles was reported in the literature, matching the results of the present investigation.

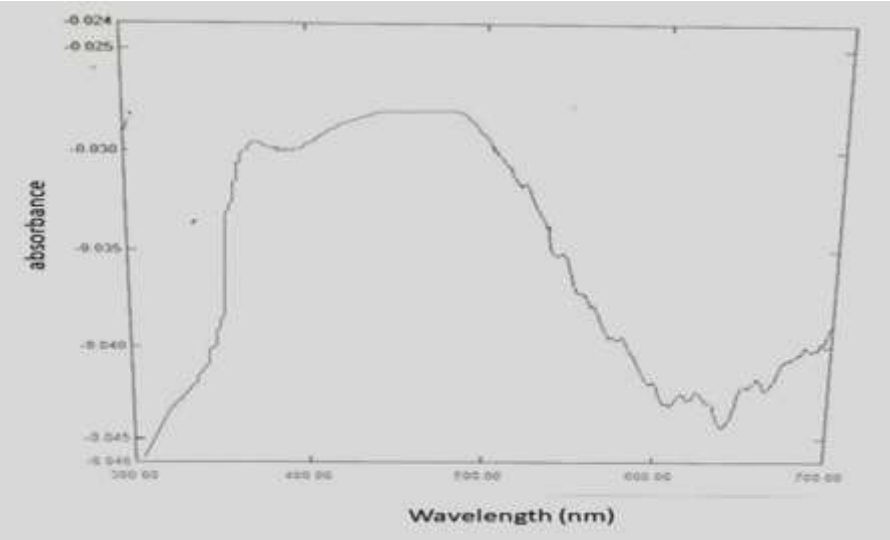


Fig. 1: Ultraviolet-visible spectrum of GG.$CuFe_2O_4$ NPs

The functional groups present in GG.$CuFe_2O_4$ NPs were detected between 4000 and 400cm1 using FTIR spectroscopy (Fig. 2). GG.$CuFe_2O_4$ NPs were found to exhibit resonance peaks at 2912, 1992, 1968, 1557, 1011, and 418cm−1 in the FTIR spectra. These peaks were characterized by the presence of Methylene (CH2) Methylene C-H asymmetry/sym. stretch, Transition metal carbonyls, Alkenyl C=C stretch, Aryl-substituted C=C Conjugated C=C, Cyclohexane ring vibrations, and Polysulfides (S-S stretch).

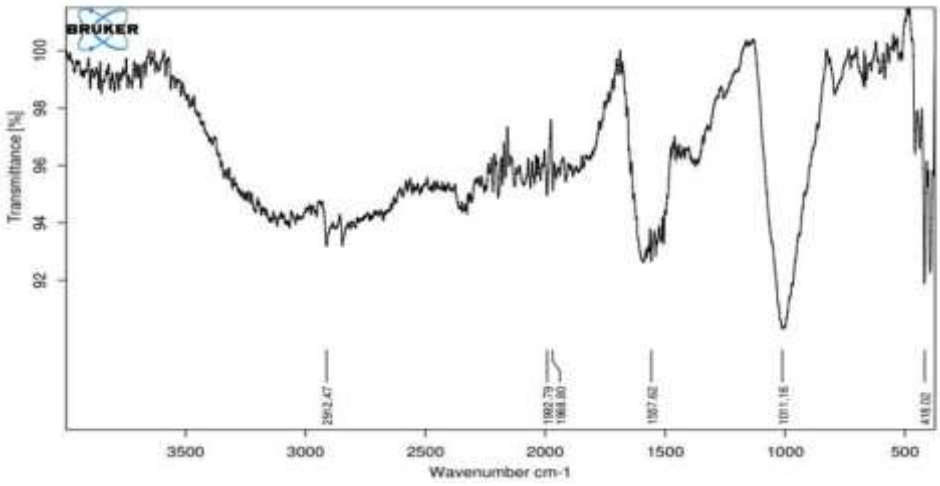


Fig. 2: Fourier transform infrared (FTIR) spectrum of GG.CuFe$_2$O$_4$ NPs

The crystal architecture of the CuO sample synthesized using the green approach utilizing the bark extract of *Glycirrhiza glabra* was determined using X-ray diffractometry (XRD) (Rigaku Smartlab 9kW Japan). Fig. 3 shows the obtained XRD pattern. The XRD pattern clearly shows the GG.CuFe$_2$O$_4$ NPs diffraction peaks at 2θ values. The crystalline character of the manufactured ferrite sample was identified by the presence of strong PXRD signals. The three main peaks were found at 2 θ = 46.240 0, 62.1080, and 78.4080. The Debey-Scherrer equation was used to determine the crystallite size, D:

$$D = 0.9 \lambda / \beta \cos \theta$$

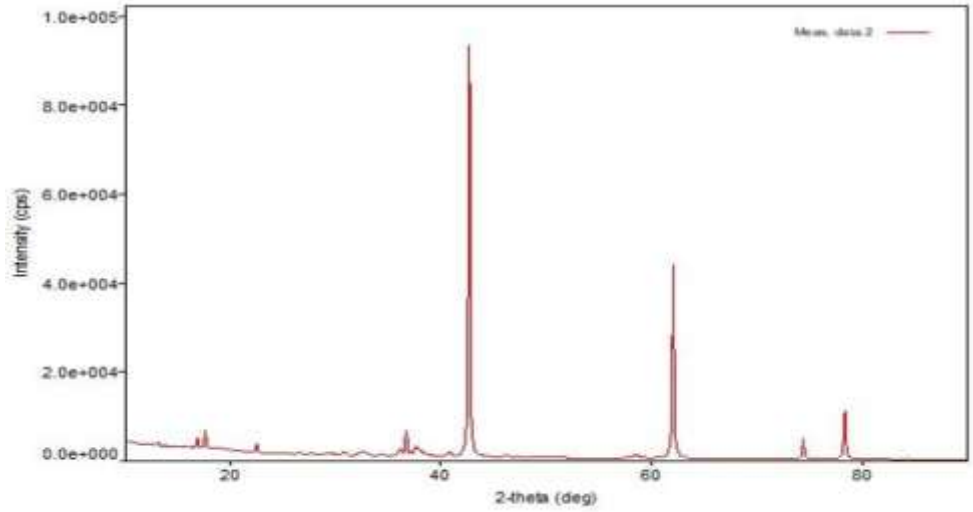


Fig. 3: X-ray diffraction pattern of GG.CuFe$_2$O$_4$ NPs at 2θ

Where β is the full-width half maximum and λ is the wavelength of the X-rays employed. The primary peak's β values were determined to be 0.42, 0.209, and 0.214. and the crystal was determined to be 22.59 lite size, D, nm. Using a Hitachi 3400SU SEM (available at Poornayu Research Labs, Nagarabhavi, Bangalore), the surface morphology and particle dispersion of the green-synthesised cupper ferrite, GG.CuFe$_2$O$_4$ NPs, calcinated at 6000C, were examined. With an accelerating voltage of 10.00kV and magnifications of 5.5mmx1.00k, 5.5mmx2.00k, 5.5mmx3.00k, 5.5mmx4.00k, and 5.5mmx6.00k at scale bars of 5.00µm, 5.00µm, 10.00µm, 2.00µm, and 50.00µm (Fig. 4). The produced nanoparticles were found to have morphological characteristics like crystals.

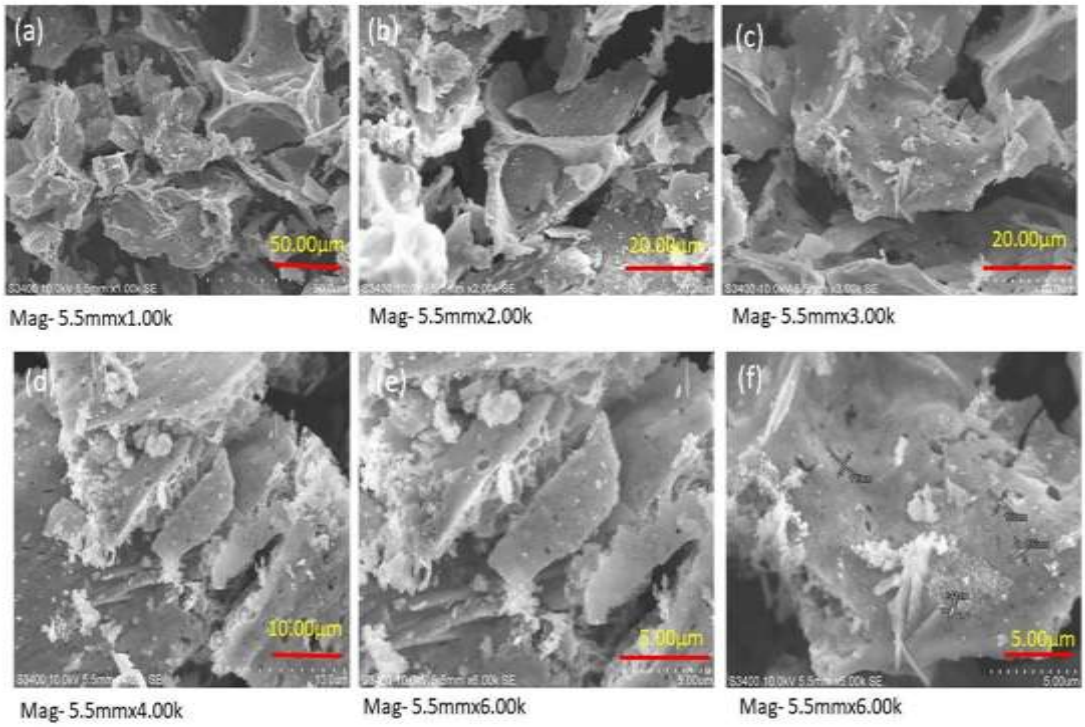
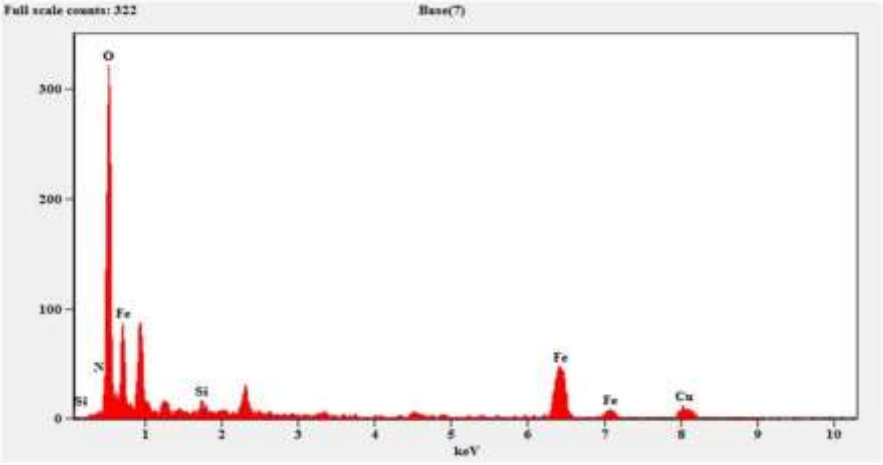


Fig. 4: Scanning electron microscope image of GG.CuFe$_2$O$_4$ NPs

EDAX (Thermoscientific, Noran, USA) was used for the elemental analysis of the GG.CuFe$_2$O$_4$ NPs. The sample's EDAX spectrum is shown in Fig. 5. Cu, Fe, and O have peaks in the EDAX spectrum. There includes trace amounts of Si in addition to Cu, Fe, and O. Cu, Fe, and O are present in the EDAX spectrum of GG.CuFe$_2$O$_4$ NPs. The effective production of copper ferrite is demonstrated by GG.CuFe$_2$O$_4$ NPs. Table 1 shows the elemental composition of GG.CuFe$_2$O$_4$ NPs. GG.CuFe$_2$O$_4$ NPs Raman spectrum is displayed in Fig. 6. A lesser peak at 236 cm−1 and a sharp peak at 709 cm−1 are also seen. Based on UV-vis, FTIR, EDEX, Raman, and XRD spectroscopy, these peaks were identified. It is established that the GG.CuFe$_2$O$_4$ NPs are primarily composed of copper and iron with trace amounts of silicon. Because the extract contains physiologically active substances that act as stabilizing factors during the nanoparticle manufacturing process, these morphological structures have been well-formed [33 & 34].



tableFig. 5: Energy dispersive analysis X-ray analysis of GG.CuFe$_2$O$_4$ NPs

Table 1. The stoichiometric ratio of 1:2 between Cu and Fe suggests the successful synthesis of GG.CuFe$_2$O$_4$ NPs. The Cu, Fe and O minor amount of Si represent the elemental composition of synthesized nanoparticles

Element Line	Weight %	Weight % Error	Atom %
C K	0.00	---	0.00
N K	2.41	±1.14	4.36
O K	45.93	±1.44	72.65
Si K	1.41	±0.24	1.27
Si L	---	---	---
Fe K	31.28	±1.44	14.17
Fe L	---	---	---
Cu K	18.96	±2.89	7.55
Cu L	---	---	---
Total	100.00		100.00

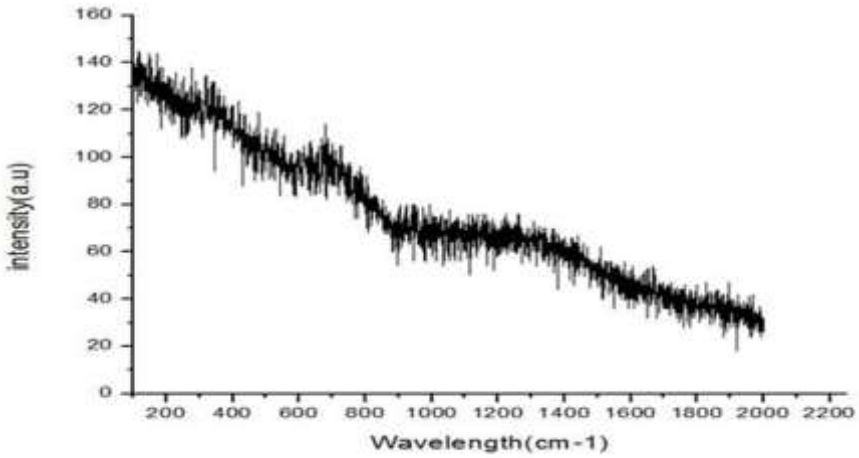


Fig. 6: Raman spectra of GG.CuFe$_2$O$_4$ NPs

GG.CuFe$_2$O$_4$ NPs exhibits antioxidant activity by Scavenging H_2O_2 and FRAP Radicals

In comparison to the positive control ascorbic acid (85%), the sample concentration of 50µg of GG.CuFe$_2$O$_4$ NPs also scavenged 80.00% of H_2O_2 radicals (Fig.7A). Furthermore, compared to the normal FRAP value of ascorbic acid (77.66%), 50µg of GG.CuFe$_2$O$_4$ NPs showed ferric-reducing characteristics with a reducing power of 53.33%. (Fig.7B). The term "oxidative stress" describes the effects of producing too many reactive oxygen species, which can harm proteins, lipids, and DNA in cells. An imbalance between the generation of oxidants and the elimination of oxidant species by endogenous antioxidant defenses leads to oxidative stress [35]. Antioxidant and anti-inflammatory properties were determined utilizing the hydrogen peroxide radical scavenging assay and the albumin denaturation inhibition assay. Zinc oxide nanoparticles made with grape seed extract demonstrated the highest level of radical scavenging activity, suggesting significant antioxidant potential [36].

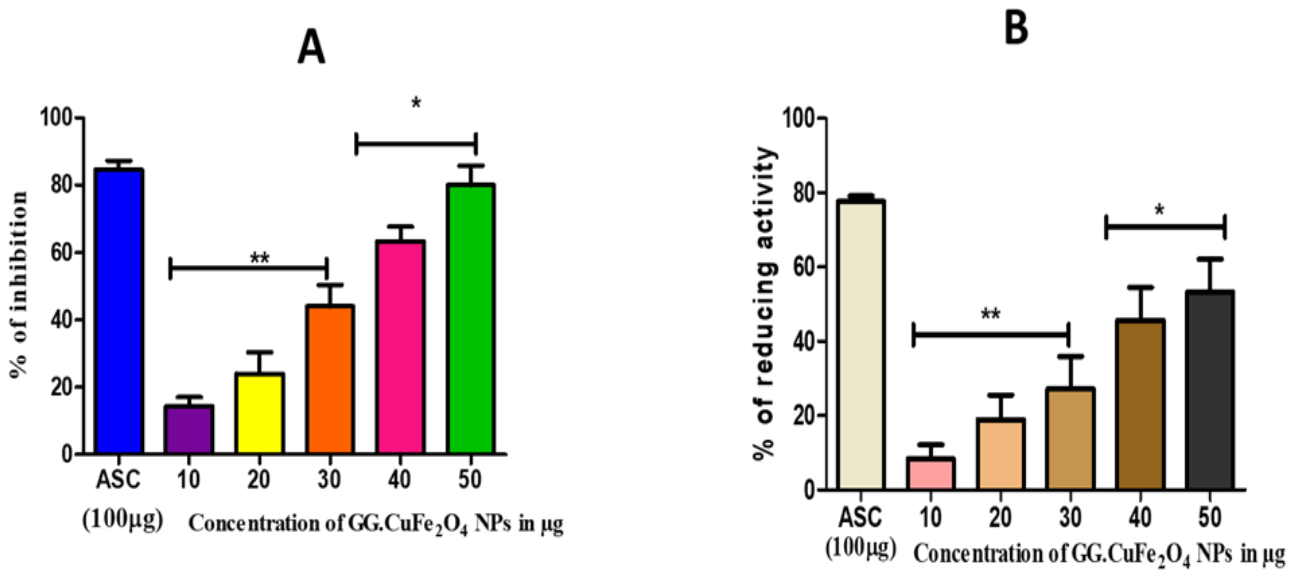


Fig. 7: Antioxidant activity GG. CuFe2O4 NPs. Antioxidant activity GG. CuFe2O4 NPs. (A) H2O2 assay. (B) FRAP assay. Each value is given as a mean ± SD. *significance at P ≤ 0.05 and * at P ≤ 0.0001. ASC: Ascorbic acid**

Anti-haemolytic activity of GG.$CuFe_2O_4$ NPs

RBCs are extremely susceptible to peroxidation and free radical damage because of the massive buildup of hemoglobin and polyunsaturated fatty acids (PUFA). During respiration, erythrocytes are regularly subjected to high oxygen pressure, which damages their structural integrity through oxidative stress [37]. Surprisingly, RBCs pre-incubated with 400µg of GG.$CuFe_2O_4$ NPs were used in a direct hemolytic assay to determine the harmful effects of GG.CuFe2O4 NPs towards the RBCs. While water was used as a positive control, GG.$CuFe_2O_4$ NPs did not lyse or disintegrate the erythrocyte suspension (Fig. 8).

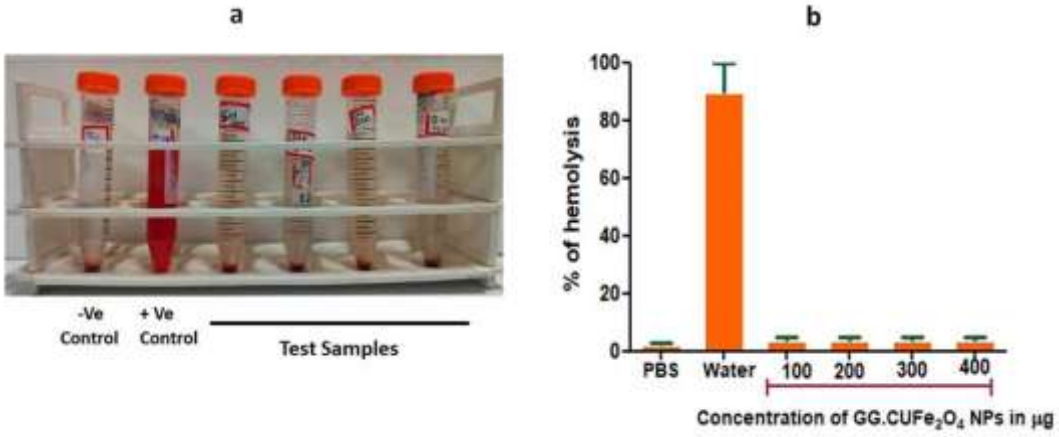


Fig.8 : Direct hemolytic assay. (A). Pictographic representation of GG. CuFe2O4 NPs showing RBC hemolytic assay. (B) The percentage of hemolysis was determined as the measure of amount of hemoglobin released.

Anti-inflammatory property of GG.$CuFe_2O_4$ NPs

The ability of a drug or therapy to lessen inflammation in the body is known as anti-inflammatory activity. The body's reaction to harm or danger is inflammation, which can result in redness, swelling, discomfort, and heat. Uncontrolled inflammation can harm tissues and organs, even though it can be

advantageous to some extent [38]. It's interesting to note that GG.$CuFe_2O_4$ NPs at 50µg protected the human red blood cell membrane against heat-induced lysis with 53.40% inhibition, while acetylsalicylic acid, a positive medication, showed 61.54% inhibition of lysis against the detrimental effect of heat solution (Fig. 9A). Additionally, the egg albumin denaturation assay is predicated on the notion that anti-inflammatory compounds might be able to stabilize protein structures and stop denaturation, which is often associated with tissue and inflammation. Denaturation of proteins is known to promote inflammation. Denatured proteins are no longer involved in biological processes [40]. The denaturation assays for egg albumin and bovine serum albumin were used to measure GG.$CuFe_2O_4$ NPs. Interestingly, the results showed that GG.$CuFe_2O_4$ NPs at a concentration of 100µg inhibited egg albumin denaturation with a percentage of inhibition of 68.73%. This was in contrast to the acetylsalicylic acid (74.66%) positive control (Fig. 9B) and 500µg of GG.$CuFe_2O_4$ NPs reduced the denaturation of bovine serum albumin by 78.63% as opposed to 83.23% for the positive control acetylsalicylic acid (Fig. 9C). CuO-NPs are expected to have significant anti-inflammatory effects since they suppress prostaglandin, serotonin, and histamine. The plant extract from Cissus arnotiana facilitated the copper nanoparticles. has antioxidant and antibacterial qualities [41]. Adhatoda Vasica was used to create copper nanoparticles, which showed anti-inflammatory and antioxidant properties. [42].

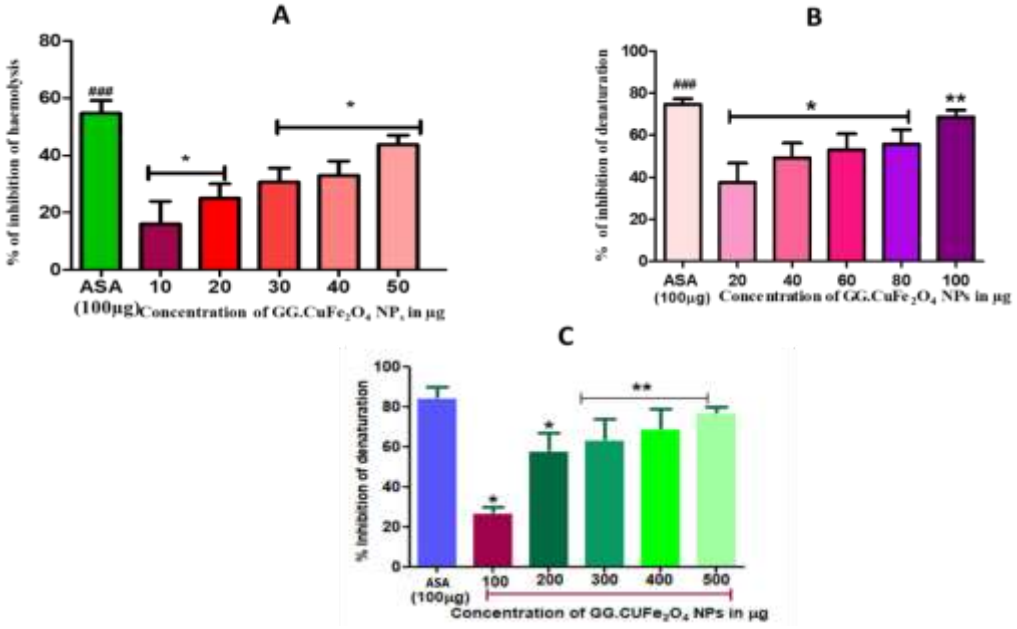


Fig. 9: The effect of GG. CuFe2O4 on inflammation. A. Heat-induced hemolysis, the percentage inhibition of RBC hemolysis. B. Egg albumin denaturation assay, the percentage inhibition of protein denaturation. C. BSA denaturation assay, the percentage. ASA: AcetylSalicylic Acid.

GG.$CuFe_2O_4$ NPs prevent λ-DNA damage

The GG.$CuFe_2O_4$ NPs demonstrated DNA protection in the present investigation due to oxidation brought on by UV light. The results shown in Figure 10 reveal that λ DNA in the presence of GG.$CuFe_2O_4$ NPs exhibited an increase in band intensity following UV exposure. where λ DNA is exposed to UV light without GG.$CuFe_2O_4$ NPs. In a 1% agarose gel, CuFe2O4 NPs displayed no band. Furthermore, GG.$CuFe_2O_4$ NPs alone did not change the integrity of λ DNA, indicating that the extract is not harmful to λ DNA. GG.$CuFe_2O_4$ NPs ability to preserve DNA from harm. Antioxidant compounds found in the green produced nanoparticle were characterized by GG.$CuFe_2O_4$ NPs. The copper nanoparticles made with extract from *Adhatoda vasica* exhibit Their strong antioxidant activity, UV-blocking qualities, and anti-inflammatory actions make them promising for photoprotective applications [43].

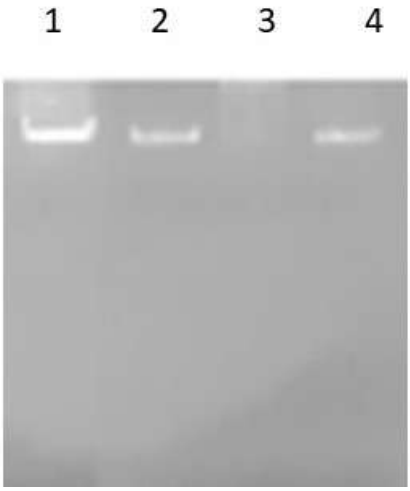


Fig. 10: 1% Agarose Gel electrophoresis image of λ DNA damage inhibition by GG. $CuFe_2O_4$ NPs. After 1 h of incubation in UV irradiation. Lane 1: 0.5 µg DNA alone; Lane 2: GG. $CuFe_2O_4$ NPs Pretreated 0.5 µg DNA; Lane 3: UV irradiated 0.5 µg DNA; Lane4: GG. $CuFe_2O_4$ NPs Pretreated (30 minutes) 0.5 µg DNA+ UV irradiation (60 minutes).

Antidiabetic activity of GG.$CuFe_2O_4$ NPs

The effects of different concentrations of GG.$CuFe_2O_4$ NPs (10 to 30µg) on the absorption of glucose in yeast cells in a 5mM glucose were examined. One of the main characteristics of diabetes is impaired cell uptake of glucose, and insulin-sensitizing antihyperglycemic medications work by helping insulin promote glucose distribution [44]. At a concentration of 30µg, the GG.$CuFe_2O_4$ NPs enhanced the absorption of glucose in yeast cells by 61% (Fig. 11). Metformin, the usual medication, has a 73.03% at 500µg. It is widely accepted that a facilitated diffusion mechanism is how glucose is delivered in yeast cells (Saccharomyces cerevisiae), despite the fact that this process is incredibly complicated. Certain carriers that move solutes along the concentration gradient are known as facilitated carriers [45].

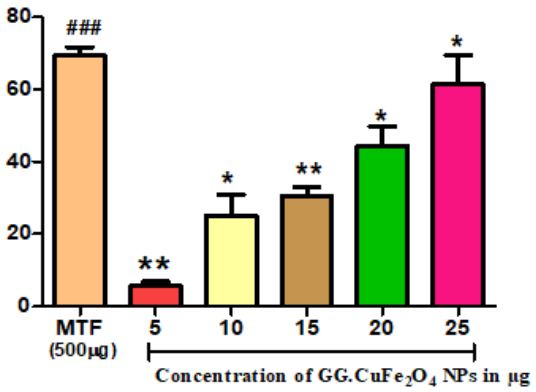


Fig. 11: Antidiabetic property of GG. $CuFe_2O_4$ NPs. Glucose uptake by yeast cells at 5mM initial concentration of glucose in the presence of GG. $CuFe_2O_4$ NPs. MTF: Standard drug MeTFormin.

GG.CuFe2O4NPs antiurolithiatic activity

The nucleation of calcium oxalate crystals was determined the nucleation inhibition 58.33% at the concentration of 25µg of GG.$CuFe_2O_4$ NPs and the cystone used as a positive control (76%). (Fig. 12). A physiological anomaly known as urolithiasis causes stones to form in the kidney, bladder, or ureter, among other parts of the urinary system. Urine supersaturation, crystal nucleation, growth, accumulation, and translocation to the renal epithelium's surface are all factors in the development

of urinary stones [45]. Renal calculi have been linked to an imbalance between stone-inhibitors (citrate, magnesium, nephrocalcin, and urine prothrombin fragment I) and stone-promoters (albumin, oxalate, and uric acid). This study's nucleation assay validates its application in assessing antiurolithiatic activity by accurately simulating the early phases of kidney stone formation [47].

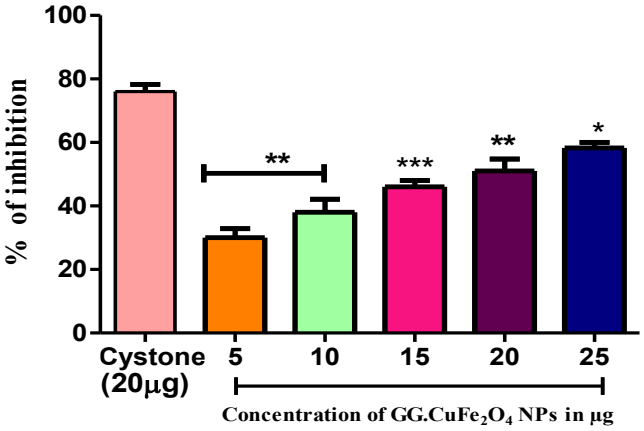


Fig. 12: Antiurolithiatic activity of GG.CuFe$_2$O$_4$ NPs. The nucleation assay employed in reliably mimics the initial stages of kidney stone formation.

Anticoagulant activity of GG.CuFe2O4 NPs

In comparison to positive (200s to 1950s) control heparin sodium, GG.CuFe$_2$O$_4$ NPs increased the clotting time of citrated human PRP from control 306s to 2400s and inhibited blood coagulation, reaching saturation at a maximum concentration of 2400µg (Fig. 13). The anticoagulant effect of GG was suggested by the considerable increase in the plasma recalcification time of citrated human plasma caused by GG.CuFe$_2$O$_4$ NPs. The body's natural reaction to stop a person from bleeding to death is clotting. However, if blood clots form in healthy blood vessels or do not break down over time, they can be hazardous. Inappropriate blood clot development is linked to numerous illnesses, including heart attack, stroke, and pulmonary embolism [48].

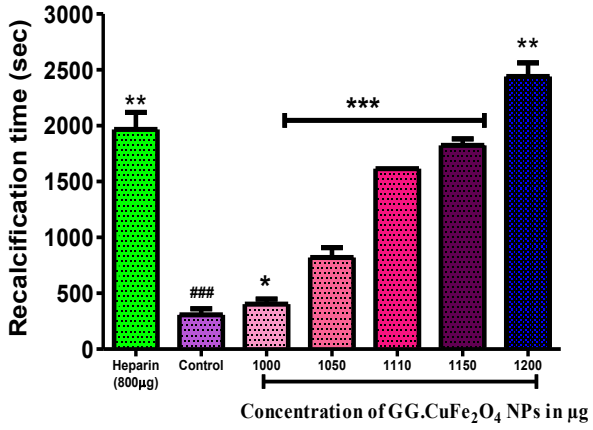


Fig. 13: Anticoagulant activity. Plasma re-calcification time. GG.CuFe$_2$O$_4$ NPs (1000–1200µg) was pre-incubated with 0.2ml of citrated human plasma in the presence of 20µl 10mM/l Tris–HCl buffer (pH 7.4) for 1min at 37°C and 20µl of 0.25mol/l $CaCl_2$ was added to the pre-incubated mixture then clotting time was recorded.

Antithrombotic activity of GG.CuFe$_2$O$_4$ NPs

GG.CuFe$_2$O$_4$ NPs hydrolyzed whole blood clot. When whole blood clot was incubated with 200 to 600µg of GG.CuFe$_2$O$_4$ NPs in a dose dependent manner, clot dissolving efficiency that was compared with the positive control streptokinase (60U/ml) and water taken as a negative control showed negligible clot lysis percentage. The percentage of clot lysis was determined to be 86% at the concentration of 600µg of GG.CuFe$_2$O$_4$ NPs against the positive control streptokinase 66.8 % (Fig. 14). Coronary artery thrombosis has been treated by synthetic drugs such as urokinase (UK), streptokinase (SK) or tissue plasminogen acti-vators (t-PA) which are widely used for the treatment of severe or massive deep venous thrombosis, pulmonary embolism, myocardial infarction, and occluded intravenous or dialysis cannulas [49]. Herbal drugs are widely used as green medicine for their safe and dependable health care. The traditional herbal medicines rise the interest since couple of decades due to their improbable pharmacological activities, economic feasibility and their less side effects [50].

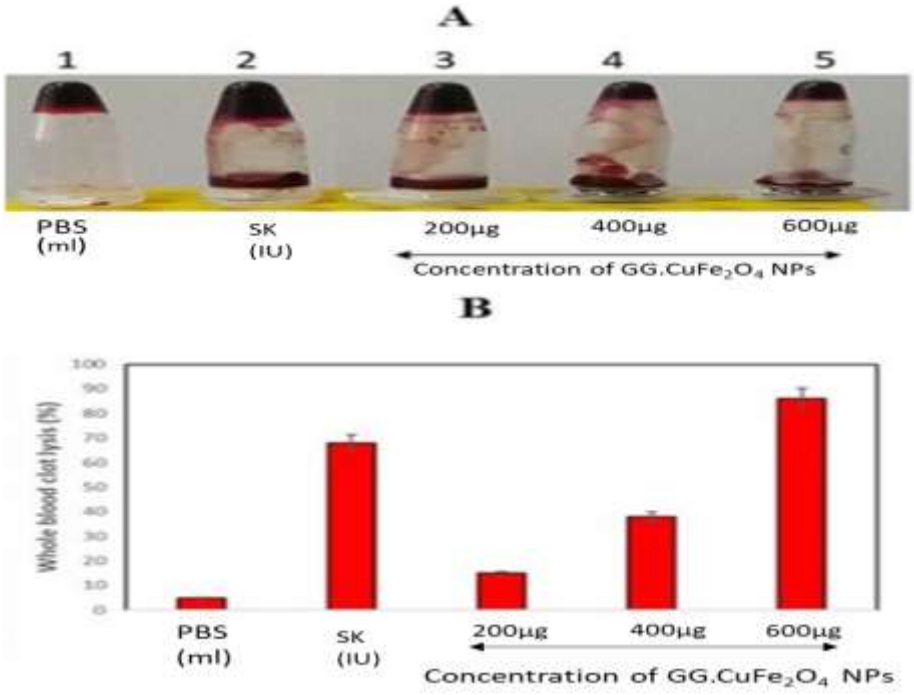


Figure 14. Whole Blood clot lysis (A) Venous blood was transferred to different pre-weighed sterile microcentrifuge tubes (500µl/tube) and incubated at 37°C for 45min. After clot formation, serum was completely removed and each tube with clot was again weighed. The 200 to 600µg of GG. CuFe$_2$O$_4$ was added to the microcentrifuge tube containing clots. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. (B) Percentage of clot lysis. (B) After incubation, the obtained fluid was removed and tubes were again weighed and blood and plasma clot lysis percentage was calculated

CONCLUSION

This present study was concluded that one of the highly medicinal value plant *Glycirrhiza glabra's* bark extract was used in this study to successfully produce copper ferrite nanoparticles, offering a low-cost, simple, and effective method for doing so. The GG.CuFe$_2$O$_4$ NPs were characterized by UV–Vis spectroscopy, FTIR, XRD, SEM, EDEX and Raman spectrophotometry. In additional anti-haemolytic, Anti-oxidant, Anti-inflammatory, Antidiabetic, Antiurolithiatic, Anticoagulant and Thrombolytic and λ-DNA damage protection activities of the biosynthesized GG.CuFe$_2$O$_4$ NPs were accessed.

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Author contributions

All authors read and approved the final manuscript.

Data Availability

Data available in corresponding author

Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

Consent to participate

All authors meet the qualifcations for authorship and had an opportunity to read and comment the manuscript.

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