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Green Synthesis, Characterization and Antibacterial Activity of Cuprous Oxide Nanoparticles Produced from Aloe Vera Leaf Extract and Benedict's Solution

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ABSTRACT

Exploring of better alternatives for antibiotics with a nano technological flavor has been recently aroused the interest of scientists. Aim of this study was to introduce an eco-friendly, reproducible, rapid and effective alternative antibacterial agent by green synthesis of cuprous oxide Nanoparticles. In this study, cuprous oxide nanoparticles were green synthesized for the first time from a reaction between Benedict's solution and Aloe vera leaf extract. The synthesized cuprous oxide Nanoparticles were characterized by different analytical techniques including UV-Visible spectroscopy, Fourier transform spectroscopy, Scanning electron microscopy, Energy dispersive X-ray spectroscopy analysis, X-Ray diffraction & Particle size analyzer. Synthesized particles were crystalline in nature and arranged as agglomerated nanospheres with an average size of 5-10 nm. According to the antibacterial potentiality assays of prepared cuprous oxide nanoparticles, the synthesized cuprous oxide nanoparticles have high antibacterial activity against Escherichia coli and Staphylococcus aureus even at lower concentrations (0.3 mg/µl- 0.6 mg/µl). According to the results of the present study, it was concluded that this green synthesized cuprous oxide nanoparticles could be proposed as a powerful eco-friendly, reproducible, rapid and effective antibacterial agent against Escherichia coli and Staphylococcus aureus.

Keywords: Aloe Vera, Antimicrobial Activity, Cuprous Oxide Nanoparticle, Green Synthesis.

1. INTRODUCTION

Finding safe and cost effective alternatives for conventional antimicrobial agents is crucial, as most of the bacteria now have been developed resistant to against those bactericides and antibiotics. There is a greater abundance of nanoparticles using as an antibacterial agent due to extreme small size and larger surface to volume ratio as it can closely interact with bacterial membrane besides releasing of metal ions. Those metal nanoparticles are combined with polymers or coated onto surfaces and act as effective antimicrobial agents in the applications of water treatment, food processing, synthetic textiles, biomedical and surgical devices [1]. The antibacterial effect of metal nanoparticles such as Copper, Silver, Aluminum, Gold, Magnesium, and metal oxides like Zinc oxide, copper oxide have been studied in previous efforts [2].

There are only few literatures on the antibacterial effect of Copper oxide nanoparticles. However, copper oxide nanoparticles are cheaper compared with Silver nanoparticles, it easily mix with polymers, relatively stable in chemical and physical properties. Being a highly ionic nano particulate metal oxide, it has significant antibacterial properties, as it can be prepared with extremely high surface areas and unusual crystal morphologies[1]. Metal nanoparticles synthesized from various chemical and physical techniques may have some limitations when

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using in clinical and biomedical applications due to its chemical and physical processes that often use toxic materials with potential hazards such as environmental toxicity, cytotoxicity, and carcinogenicity. Elsewhere, all most all these methods are complex, lengthy, eco-hazardous and energy intensive routes, which require cumbersome and expensive material [3]. In this paper, we suggest an eco-friendly method of synthesizing cuprous oxide nanoparticles to be used as an antibacterial agent for the first time by using *Aloe vera* plant extract with the Benedict's solution.

Aloe vera (Aloe barbadensis Mill.) is a well-known medicinal herb, which comprises nearly 75 potentially active ingredients having immunomodulatory, antiparisatic, wound healing and antimicrobial potential [4]. *Aloe vera* gel itself have shown antibacterial and antiviral properties as it contains Anthraquinones [5]. Biomolecules, reducing sugers, secondary metabolites, phytochemicals such as amino acids, proteins, polysaccharides, vitamins, polyphenols and terpinoids present in *Aloe vera* act as natural bio reductants, stabilizing and capping agents and can be used to replaces the toxic chemicals use in other chemical and physical methods of synthesizing nanoparticles. Therefore, these plant-mediated nanoparticles have great potential in clinical and biomedical applications. Antibacterial effect of the synthesized cuprous oxide nanoparticles was studied against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) after confirming the formation by characterization from several analytical techniques.

2. MATERIAL AND METHODS

According to literature, Cu_2O has been synthesized from Fehling's and Barfoed's solution which are commonly used for identification of reducing sugars [3][6]. Benedict's solution, which is used to identify reducing sugars, has been used during this study to synthesize cuprous oxide by reacting with *Aloe vera* leaf extract.

Aldehyde groups of carbohydrates are oxidized to carboxylic acid group while reducing other metal ions like Copper (II) ions or silver (II) making Copper (I) and Silver respectively. Attraction of electrons from the aldehyde or ketone group of reducing sugars in Aloe vera extract by the copper (II) ions in the Benedict's solution change it to copper (I) which then precipitates as cuprous oxide. The chemical reaction that occurs can be shown in the following equation:

$$RCHO + 2Cu^{+2} + 2H_2O \rightarrow RCOOH + Cu_2O + 4H^{+} \tag{1}$$

2.1 Preparation of Benedict's Solution

50 ml of Benedict's solution was prepared by mixing of 0.865 g of Copper sulphate, 5 g of Sodium carbonate, and 8.65 g of Sodium citrate and diluted up to 50 ml of distilled water. (All the chemicals were analytical grade).

2.2 Preparation of Aloe Vera Leaf Extract

25 g of clean, dry *Aloe vera* leaves were finely chopped & boiled for 5 min, at 80 ^{0}C on a hot plate with 100 ml of distilled water in a 250 ml Erlenmeyer flask and cooled down to room temperature. The resulting solution was filtered through filter papers (double ring filter paper no. 102) to remove solid particles. The filtrate was stored at 40 ^{0}C as a stock for the synthesis of cuprous oxide nanoparticles.

2.3 Green Synthesis of Cuprous Oxide Nanoparticles

Aloe vera solution was added dropwise to the prepared 50 ml of Benedict's solution in 3min interval in a boiling water bath at 90 ^{0}C. When adding, Benedict's solutions change its blue color to green and then to yellow brown and transformed into brick red. This experiment was conducted three times giving same experimental conditions. In first, a second and third experiment, the reaction was stopped when it was turned turn green, yellow brown and brick red respectively. Each resultant was centrifuged separately at 10,000 rpm for 10 min, at room temperature and the bottom particles were kept at room temperature until the evaporation of aqueous phase to collect the nanoparticles.

2.4 Characterization of Synthesized Cuprous Oxide Nanoparticles

The synthesized cuprous oxide nanoparticles were characterized by different analytical techniques including UV-Visible spectroscopy (SHIMAZU 1800 UV), Fourier transform spectroscopy (SHIMADZU IRAffinity) and scanning electron microscopy (SEM) Zeiss Evo LS15, Energy dispersive X-ray spectroscopy analysis (EDX) Oxford Instruments INCA X-Act, X-Ray diffraction (XRD) Rigaku ultima IV & Particle size analyzer (FRITSCH,ANALYSETTE 22,NanoTec plus).

2.5 Antibacterial Activity of Green Synthesized Cuprous Oxide Nanoparticles

Antibacterial activity of synthesized nanoparticles were analysed against two common human pathogenic bacteria, *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) by disk diffusion method. Nanoparticles were dispersed in sterile distilled water and prepared 0.2, 0.3, 0.4, 0.5 and 0.6 mg/µl cuprous oxide solutions. 5 µl of each nanoparticle solutions (0.2, 0.3, 0.4, 0.5, and 0.6 mg/µl) were loaded onto autoclaved filter paper discs (double ring filter paper no. 102, 5 mm diameter) and those paper discs were allowed to air dry. A loopful from *Escherichia coli* and *Staphylococcus aureus* culture samples were sub cultured on two separate Müller-Hinton agar (MHA) plates using an inoculating loop and the dry discs were placed on the previously inoculated Müller-Hinton agar medium. In this study, Tetracycline was used as a positive control and *Aloe vera* leaf extract and Benedict's solution were used as negative controls. This antibacterial sensitivity test was conducted in three replicates. Zone of inhibition of bacteria was measured after incubation at 37 ^{0}C for 24 hours. All the required glassware, culture media and pipette tips were used after autoclaving at 121 ^{0}C for 20 min.

3. RESULTS AND DISCUSSION

UV-VIS spectroscopy was used for the confirmation of the synthesis of nanoparticles and examine the size and shape controlled nanoparticles in aqueous solution. Figure 1 shows UV-VIS spectra for (a) Benedict's solution, (b) *Aloe vera* extract, (c) colloidal suspension after converted to Green color (d) colloidal suspension after converted to yellow brown color (e) colloidal suspension after converted to brick red color when mixing *Aloe vera* leaf extract with Benedict's solution. UV-VIS spectra for colloidal suspensions of green, yellow brown and brick red samples shows the characteristic absorption peak at 388 nm, 399 nm and 401 nm respectively due to surface plasmon absorption of metal oxide (Fig 1) [6]. The observed red shift implies that the particle size increases with the color change from green to brick red. Particle size of green, yellow brown and brick red was recorded as 90 nm, 1.51 µm and 2.10 µm respectively by the particle size analyzer (Fig 2). Therefore, it is confirmed the particle size increment with the color change from green to brick red when mixing with *Aloe vera* leaf extract with Benedict's solution.

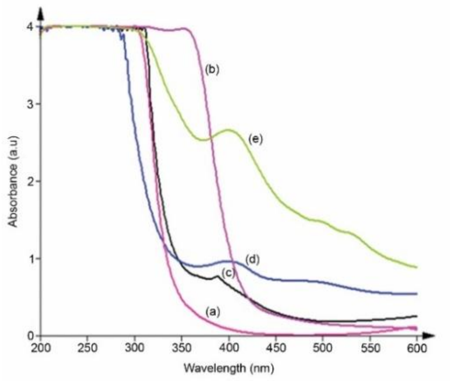

Figure 1. UV-VIS spectra for (a) Benedict's solution, (b) *Aloe vera* extract, (c) colloidal suspension after converted to Green color, (d) colloidal suspension after converted to yellow brown color and (e) colloidal suspension after converted to brick red color.

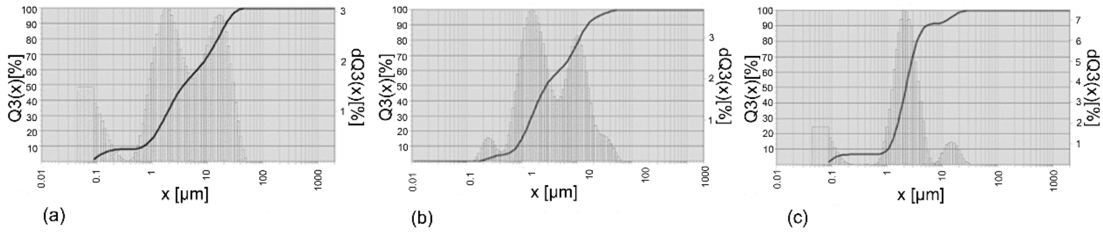

Figure 2. Histogram of the particle size distribution in (a) Green color colloidal suspension (b) yellow brown colloidal suspension (c) brick red color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution.

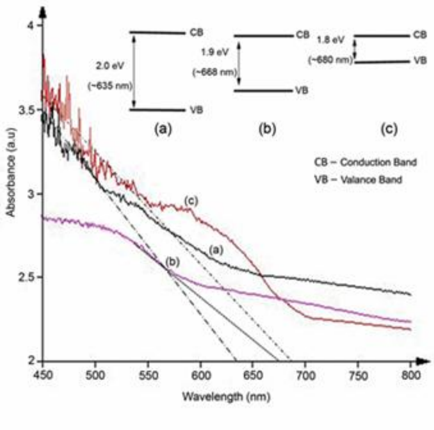

Figure 3. Diffuse reflectance spectra for (a) cuprous oxide particles taken from centrifuging green color colloidal suspension, (b) cuprous oxide particles taken from centrifuging yellow brown color colloidal suspension and (c) cuprous oxide particles taken from centrifuging brick red color colloidal suspension.

Figure 3 shows the diffuse reflectance spectra of the particles taken after centrifuging green, yellow brown and brick red colloidal suspension. The bandgap measurement for the synthesized particles was calculated by using the formula $E_g = hc/\lambda$ where h is the Planck's constant, c is the speed of light and λ is the absorption edge of the diffuse reflectance spectra [7]. The direct band gap (Eg) value was observed to be ~2.0 eV, ~1.9 eV, ~1.8 eV and schematically shown in a, b, and c respectively as shown in Figure 3. Formation of Cuprous oxide was further confirmed by these band gap measurements as it is a well-known semiconductor material with theoretical direct band gap of ~2.0 eV [3]. Moreover, direct band gap values were observed to be decreased from a to c may be due to quantum confinement effect [7].According to the above results from the Diffuse reflectance spectra and histogram of the particle size distribution it was revealed that the particle size was increased when the mixture changes its color from blue to brick red. Therefore, it was suggested to stop dropwise adding of *Aloe vera* leaf extract to the Benedict's solution when it converts from blue to green. Particles taken from centrifuging the green colour colloidal solution were used for further characterization by scanning electron microscopy (SEM), energy dispersive x-ray (EDX) analysis, Fourier transform infrared (FTIR) spectroscopy and X- ray diffraction patterns (XRD).

The surface morphologies and the elemental analysis for synthesized nanoparticles in Green color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution were described from scanning electron microscopy (SEM) images and energy dispersive x-ray (EDX) analysis and the results are shown in the Figure 4. According to the scanning electron microscopy in Figure 4(a), it was identified that agglomerated nanospheres with an average size of 5-10 nm. This agglomeration of nanospheres may be interpreted in terms of the increase in the catalytic activity of the surface of the nanoparticles. Thus, it can be predicted that these green synthesized nanoparticles have a great potentiality as a powerful antibacterial agent. EDX profile as in Figure 4(b) of synthesized cuprous oxide particles shows strong copper signal along with oxygen and weak carbon peak confirming the formation of cuprous oxide nanoparticles.

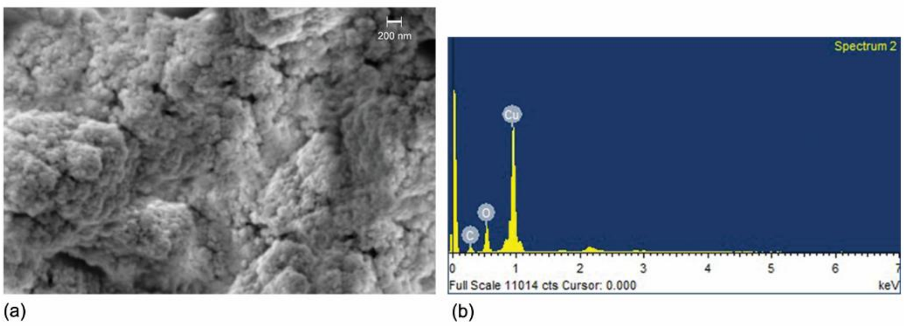

Figure 4. (a) SEM image (b) EDX Profile taken from the cuprous oxide particles in green color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution.

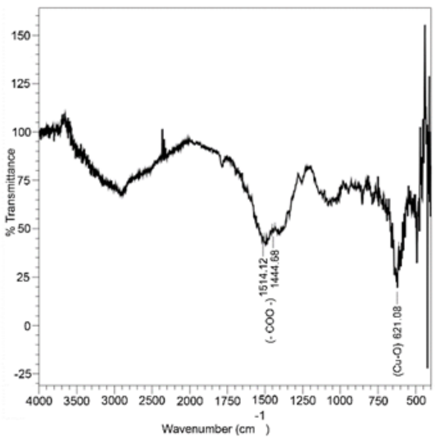

Figure 5. FTIR spectra of green synthesized cuprous oxide nanoparticles in green color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution.

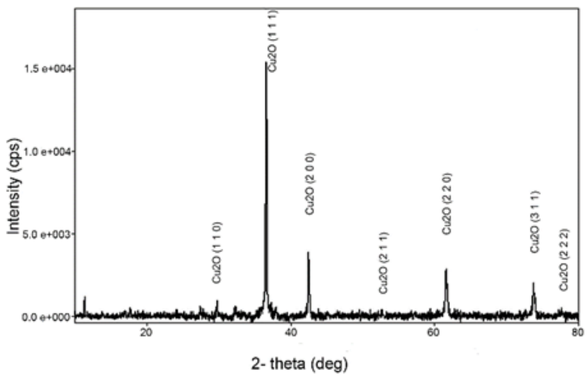

Figure 6. XRD spectrum of cuprous oxide nanoparticles synthesized in Green color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution.

Fourier transform infrared (FTIR) spectroscopy of synthesized cuprous oxide nanoparticles in Green color colloidal suspension was done using KBr pallet method in the range 4000-500 cm^{-1} in order to identify the functional groups of synthesized nanoparticles. According to the results (Fig 5) peaks were observed at 1514.12 cm^{-1}, 1444.68 cm^{-1} and 621.08 cm^{-1}. The strong peak at 621.08 cm^{-1} indicates that the formation of cuprous oxide nanoparticles as the presence of cuprous oxide can be confirmed by the detection of peaks in the range of 605 to 660 cm^{-1} in FTIR spectrum [8]. It is well consistent with the results of previous studies as well [3][6][9]. The Peak at 1514.12 cm^{-1} and 1444.68 cm^{-1} indicate the presence of (-COO-) carboxylate ions, responsible for stabilizing the cuprous oxide nanoparticles [6]. If cupric oxide is present in the sample, it should indicate three major vibrational peaks in the range 500-700 cm^{-1} [10]. However, the FTIR spectrum results exclude the presence of cupric oxide impurities, further confirming the formation of high pure cuprous oxide nanoparticles.

Crystal structure of synthesized cuprous oxide nanoparticles in green color colloidal suspension when mixing *Aloe vera* leaf extract with Benedict's solution was studied using Cu Kα(λ=1.5405 Å) radiation from Regaku Ultima IV x-ray diffractometer. Crystal phases were identified by using PDF2 database from International Center of Diffraction Data. The X-ray diffraction (XRD) patterns obtained for the synthesized cuprous oxide nanoparticles are shown in Figure 6. The XRD spectrum contains seven peaks that were clearly distinguishable. All of them can be perfectly indexed to crystalline cuprous oxide in peak position. The peaks with 2θ values of 29.698°, 36.568°, 42.487°,52.688°,61.598°,73.779° and 77.65° were corresponded to the crystal planes of (110), (111), (200), (211), (220), (222) and (311) of crystalline cuprous oxide respectively. Characteristic peaks for copper metal or cuprite structure were not detected indicating that the synthesized cuprous oxide nanoparticles were in pure form. The present experimental results were found to be in agreement with the reported diffraction patterns of cuprous oxide nanoparticles in previous studies [3][6][9].

Antibacterial effect of synthesized cuprous oxide nanoparticles were assessed by disk diffusion method. Zone of Inhibition (ZOI) for *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) was measured and the data presented in table 1 as mean ± SE (standard error). No zone of inhibition was observed for the disks filled with prepared *Aloe vera* leaf extract and Benedict's solution resulting that there was no any antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* from *Aloe vera* leaf extract and Benedict's solution alone. According to the results presented in Table 1, it can be concluded that the synthesized cuprous oxide nanoparticles have great antibacterial activity against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) even at lower concentrations (0.3 mg/µl). As nanoparticles have larger surface area, it may have better contact with microorganisms. Thus, the antibacterial activity is more efficient even in lower concentration. Zone of Inhibition for *Staphylococcus aureus* and *Escherichia coli* was noticed to be increased with increasing concentration of cuprous oxide nanoparticles. Maximum Zone of Inhibition was recorded at 0.6 mg/µl cuprous oxide nanoparticles for both bacterial species tested. Around 13.0 ± 0.8 mm of Zone of inhibition was observed for *Staphylococcus aureus*, and in the case of *Escherichia coli* it was recorded as 17.2 ± 0.8 mm at 0.6 mg/µl concentration of cuprous oxide nanoparticles. It reveals that gram-positive *Staphylococcus aureus* is less sensitive to synthesized cuprous oxide nanoparticles than gram-negative *Escherichia coli*. It is in agreement with previous studies as well [9][10][11]. The probable mechanism behind the antibacterial activity of metal oxide nanoparticles has been interpreted using several scientific explanations in previous studies. However, the exact mechanism is not fully understood to yet[1][9]. That difference of antibacterial sensitivity between gram-negative *Escherichia coli* and gram- positive *Staphylococcus aureus* may be due to the differences in composition of the bacterial cell membrane. In both gram positive and gram negative bacteria have amine and carboxyl groups in their cell walls, which have great affinity towards copper. Cuprous ion release from the cuprous oxide nanoparticles bind with negatively charge cell wall in gram-negative bacteria and rupture it. Thereby the protein inside the microorganisms would denature and can cause cell death [6][9]. However, gram-positive bacteria is having a thick peptidoglycan cell wall, it is difficult to penetrate cuprous ion inside the cell [10]. Therefore, it can be revealed that synthesized cuprous oxide nanoparticles have more antibacterial effects towards gram-negative bacteria than gram-positive bacteria. Once entered it would go and bind with DNA of microorganisms and disrupt the helical structure of it by forming cross links within and in-between DNA molecules. Furthermore, it would disrupt the biochemical process inside bacteria as well[1] [4][9]. Bacterial growth is further suppressed by indirect effect from changing the surrounding environment of bacteria by releasing cuprous ions from the nanoparticles [12]. Further studies should be carried out to find the antibacterial effect of synthesized cuprous oxide nanoparticles for various other pathogens and ensure the minimum inhibitory concentration and minimum bactericidal concentration of the synthesized nanoparticles. This method of synthesizing nanoparticles can be suggested commercially fabricate wall coverings, equipment, clothing and bedding in hospitals, which are having possible risk of spreading infection.

Table 1 Zone of inhibition (mm) of *staphylococcus aureus* and *escherichia coli* by synthesized cuprous oxide nanoparticles

	Zone of Inhibition (mm)							
	0.2 mg/μl	**0.3 mg/μl**	**0.4 mg/μl**	**0.5 mg/μl**	**0.6 mg/μl**	**Negative control *Aloe vera* leaf extract**	**Negative control (Benedict's solution)**	**Positive control (Tetracycline)**
Escherichia coli	15.6 ± 0.6	15.8 ± 0.4	16.1 ± 0.4	16.2 ± 0.3	17.2 ± 0.8	NA	NA	18 ± 0.4
Staphylococcus aureus	12.4 ± 0.8	12.5 ± 0.8	12.7 ± 0.8	12.9 ± 0.8	13.0 ± 0.8	NA	NA	16 ± 0.3

4. CONCLUSION

High purity cuprous oxide nanoparticles were prepared successfully by the reaction between *Aloe vera* leaf extract and Benedict's solution. According to results of diffuse reflectance spectra and particle size analyzer, it was suggested to stop the reaction just after observing the brick-red small particles in green colored colloidal suspension in order to obtain cuprous oxide particles with average size of 90 nm. Fourier transform infrared (FTIR) spectroscopy, X- ray diffraction patterns and EDX profile further confirmed the formation and purity of cuprous oxide nanoparticles by this novel biosynthesis method using *Aloe vera* Leaf extract and Benedict's solution. As stated by the scanning electron microscopy, it was revealed out that these nanoparticles were further agglomerated with nanospheres about 5-10 nm which can be attributed to an antibacterial effect. According to the antibacterial sensitivity test by disk diffusion method, it was found that synthesized cuprous oxide nanoparticles have a great antibacterial activity against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) even at lower concentrations (0.3 mg/μl-0.6 mg/μl). According to results obtained from the present study, these synthesized nanoparticles could be a powerful eco-friendly, rapid and effective antibacterial agent against *Escherichia coli* and *Staphylococcus aureus* and a potential use in pharmaceutical and medical applications as disinfectant agents and active ingredient for dermatological applications after confirming either its biosafety or toxicity.

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