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Bioactive Evaluations of Acetaminophen Tethered Silver Nanoparticles to Control Vector Borne Disease Mosquitoes

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Abstract: ***Acetaminophen is a non-steroidal anti-inflammatory drug that is commonly used as an analgesic and antipyretic. Due to the occurrence of side effects in human organs, several misconceptions have recently been developed about drug mechanism/toxicity. To overcome the toxicity and to increase efficacy nanotechnology is employed in the present study. Silver nanoparticles (AgNPs) were synthesized through acetaminophen drug and characterized. UV-Vis and XRD spectra provide SPR peak at 438nm and 10nm crystalline size respectively which was calculated by using intense Ag peak of 2θ=38.4, 46.3, 64.7 and 78.0º. HR-SEM and HR-TEM images showed that the AgNPs have spherical morphology with 10 nm. Biological activity was carried out with bacterial pathogens and mosquito vector and showed consistent activity. In drug releasing study, AgNPs has excellent drug releasing behavior at pH-5.0 and pH-7.4. Further in vivo studies are in progress for the development of drug for the control of human diseases.***

Keywords: Acetaminophen, AgNPs, drug discovery, mosquito vector, pathogens.

1. Introduction

Extreme climatic conditions and changing food habits create an environment for the emergence of dreadful diseases in human beings. Most of the common bacterial species have attained antimicrobial resistance in infected patients leading to increasing mortality [1]. Mosquitoes are believed to be one of the important vectors for the transmission of diseases such as dengue, chikungunya, yellow fever etc. in human beings [2]. Globally, mosquito borne disease incidence has been drastically increasing every year in topical and semitropical regions [3]. Generally, insecticide aerosols contain active pesticides that are controlled only the adult mosquitoes but are expensive and lead to detrimental side effects [4]. Controlling mosquitoes at larvae and pupa stages are more economical as they are more vulnerable and easier to eradicate rather than the adult counterparts [5]. Alternatively, natural product research for mosquito control is enormous but still it is infancy due to lack of clinical trials. Synthetic remedies are well recognized for their speedy action, but a major drawback is biomagnification and non-selective in nature [6] Acetaminophen (ACT), a synthetic drug, has been used as an antipyretic or analgesic globally since 1955; however, its purpose and safe dosage are unknown. However, as per a cross-sectional study conducted in 2011 to assess public understanding of the ATC drug, only 68 percent are aware of its use, while only 17 percent and 35 percent are aware of the medicine's side effects [7]. The ACT drug has similar analgesic property, but the mode of action is different from the other [8]. The mode of action for ACT is lowering the cyclooxygenase level in the central nervous system by inactivating cyclooxygenase in the presence of low level of peroxidise [9,10]. It was already reported that in mid-1980's ACT is considered safe with fewer side effects of liver necrosis at acute overdose [11] but no side effects when administered by appropriate dosage [12,13]. Exceptionally, the chronic dosage liver necroses have also been associated with fasting, malnutrition, alcohol consumption or concomitant administration with drugs inducing cytochrome P450 enzyme [14]. Recently, the US FDA has revised the warning label of acetaminophen toxicity, so discovery and development is needed for their derivatives [15,16]. Nanotechnology is an innovative tool for the preparation of novel medicines to control/cure various human diseases [17]. NPs provide an essential contribution to the diagnosis and management of several human diseases. The main advantage of NPs includes reduced toxicity and improved therapeutic efficacy [18]. NP drug delivery is a targeted therapy enabling the drug to be delivered at the specified site and the therapeutic concentration can be achieved at lower concentrations of the drug which in turn reduces the side effects at non target site [19]. This therapeutic efficiency of NPs thereby improves the drug's water solubility and half-life period [20]. NPs with hazardous chemicals are not suitable for drug delivery. Some of the important specific NPs including AuNPs, PtNPs, AgNPs etc exhibit super potential targeted drug delivery of therapeutic for the treatment of human diseases. Especially, AgNPs gained importance among all as silver has proven efficacy against diverse bacterial species and in wound healing since ancient period with less cytotoxicity [21]. In this context, the suitable effective delivery of NPs tethered with drug molecules improves the therapeutic strategies for various diseases [22]. Formulation of AgNPs has unique properties and gained enormous attention in biomedical fields [23]. In recent times, the in vitro drug release study gained

importance as the substitute for inexpensive vivo methods and because of its ability to predict the drug's in vivo behavior in terms of dosage, release mechanism and kinetics providing basic scientific approach for drug development [24]. The goal of this research is to create acetaminophen-tethered silver nanoparticles using a simple method and then characterize them using various sophisticated methods to confirm the formation of the SPR band, Ag nanoparticles, and functional groups. Furthermore, synthesized AgNPs were carried out the biological activity of AgNPs and its drug delivery through in vitro drug release study.

2. Materials and Methods

Acetaminophen was purchased from Sigma Aldrich. Silver nitrate was obtained from Alfa Aesar chemicals.

2.1 Synthesis of silver nanoparticles (AgNPs)

Acetaminophen powder (5g) was subjected to silver nitrate aqueous solution (50 mL 1 Mm). The mixture was kept in shaker and incubated overnight at 28°C at 100 rpm. The reaction mixture was kept under observation for 24 h to obtain the dark brown, indicating the formation of AgNPs. The colour change from light yellow to dark brown could be due to reduction of Ag+ to Ag0, which is confirmation of AgNPs formations. After 24 h, mixture was centrifuged at 6000 rpm for 10 min and supernatant was stored at refrigerated conditions for further usage [25].

2.2 Characterization techniques

The AgNPs were subjected to characterize the various analytical techniques such as UV-Vis (Shimadzu UV -2600), XRD (JEOL IDX 8030 X-ray diffractometer), Fourier transform infrared spectroscopy (BRUKER Optik GmbH-TENSOR 27 spectrometer), HRSEM (FEI quanta FEG 200 instrument) and HRTEM (JEOL JEM 2100 instrument.

2.3 Antibacterial activity

The antibacterial activity for synthesized AgNPs was assessed by zone of inhibition (mm dia.) with different concentrations (100µl, 200µl, 300µl and 400µl) by agar well diffusion method against E. coli (MTCC 41); Staphylococcus aureus (MTCC 11949); Bacillus cereus (MTCC 1306); Klebsiella terrigena (ATCC 700372); Pseudomonas aeruginona (ATCC 15442); Micrococcus mucilaginasus (ATCC 25296).

2.4 DPPH radical scavenging assay

The effectiveness of synthesized AgNPs to quench DPPH was determined using Hanato and Kagawa's (1988). After 10 minutes, the absorbance was measured at 515nm using a 0.15 percent DPPH solution mixed with serial dilutions (100-500g/mL). The antiradical activity was expressed as IC50 (ug/mL) method (the antiradical dose required to cause a 50 percent inhibition). Vitamin C was used as a reference standard and assay was analyzed in triplicate. The following equation was used to calculate the ability to scavenge the DPPH radical.

$$\text{DPPH scavenging capacity (\%)} = [(\text{Asample} - \text{Ablank})/\text{Acontrol}] \times 100$$

where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of synthesized AgNPs at 30min.

2.4 Larvicidal and pupicidal assay

The pupa and instar larvae of Aedes aegypti mosquitoes were reared in plastic tray using distilled water. One milliliter of AgNPs (20, 40, 60, 80 and 100ppm) was added in to 20 specimens of first to fourth instar larvae and pupae containing 249 ml of dechlorinated water. Quadruplicates with negative control (DMSO) were maintained. Mortality rates were subjected to statistical analysis. The $P<0.05$ level values were considered for significance[26].

2.6 In Vitro Drug Release Study

In Vitro drug release experiment was performed by dialysis bag method at two different pH values (pH = 7.4 in PBS and pH = 5.0 in acetate buffered solution) with or without esterase using pure acetaminophen and acetaminophen tethered AgNPs to investigate the drug release profile. Acetaminophen and acetaminophen tethered AgNPs were dispersed into 5 mL of buffer solution separately. Each solution was loaded into a kDa MWCO dialysis bag and then immersed into 100 mL buffer solutions at 37 °C. At the end of time point, concentration of acetaminophen in the buffer solution surrounding the dialysis bag was recorded by using UV absorbance measurement. Each time point the external buffer solution was replenished. The cumulative drug release was determined in the presence and absence of esterase (30 U/mL).

3. Result and Discussion

3.1 Characterization of Synthesized nanoparticles

Synthesis of AgNPs by the reduction of Ag ions after conjugating the acetaminophen at room temperature was recorded by UV-Vis, XRD and FT-IR spectroscopy (Figure 1 A-C). The formation of surface Plasmon resonance peak (SPR) was preliminary checked by UV-Vis spectroscopy. During the experiment, the obtained dark brown color could be due to excitation of SPR, indicating the reduction of Ag ions to Ag particles. Figure 1 A clearly shows the narrow SPR peak centered at 438 nm along with pinnacles in the range from 400 to 450 nm [27]. This pinnacle may be due to the highest population of monodisperse smaller spherical particles. Supporting affirmation for this observation is obtained from HR-SEM and HR-TEM analyses. In addition to confirming the crystalline nature and particles size of the AgNPs, XRD spectroscopy was used. Figure 1B provides four intense peaks at 2θ=38.4, 46.3, 64.7 and 78.0º which represented Bragg reflections from (111), (200), (220) and (311) planes of face-centered cubic structure of Ag (J CPDS:80-3722) [25]. The crystalline size of AgNPs is calculated from the XRD pattern by using Debye-Scherrer formula. The average crystalline size is enumerated as 10nm. Furthermore, the functional groups present in the acetaminophen were confirmed by FT-IR spectroscopy. From the analysis, there are four peaks observed such as

3280, 1642, 1454, 1043, 853 and 712 cm-1 which are assigned to the O-H, C=O, NH, C-H, C-N amide stretching respectively. The functional groups may be responsible for the reduction and stabilization of NPs. Based on the results, it is speculated that the AgNPs is successfully synthesized.

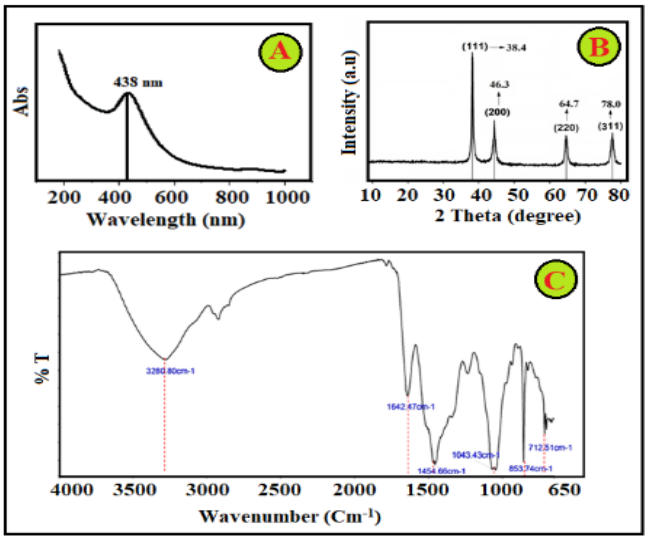

Figure 1: Characterization of synthesized nano particles.

3.2 Morphology analyses

The morphological structure of AgNPs and metal present is divulged from HR-SEM and HR-TEM analyses as illustrated in Figure 2 (A-D). Figure 1 A exhibits spherical morphology that is identically dispersed. All the spherical particles are composed of Ag elements which is affirmed by EDAX analysis (Figure 1 B). Similar morphology along with mono dispersed particles is also observed in Figure 1C. The size of the AgNPs is found to be below 10 nm in both HR-SEM and HR-TEM analyses. In SAED pattern, the white color ring is observed which is witnessed to confirm the crystalline order of the AgNPs (Figure 2D). The SEAD circular rings are correlated well with XRD pattern.

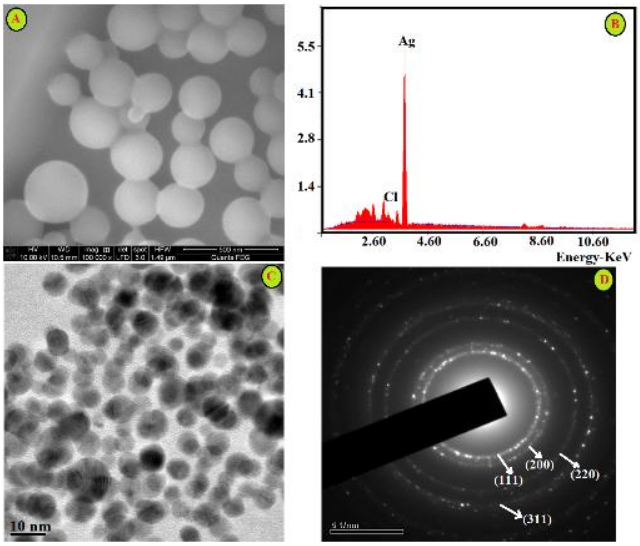

Figure 2: Morphological identification particles.

3.3 In vitro antibacterial, antioxidant, mosquitocidal and In Vitro Drug Release studies

Antibacterial activity of AgNPs is presented in figure 3 A-F. The result showed that AgNPs has a highly effective inhibition against E. coli compared to other pathogens. This may be due to interaction between thin layer cell wall and spherical NPs [27]. The NPs may release the Ag+ ion at desired location and rupture it. The antibacterial activity of AgNPs may depend on the particles size and different environmental circumstances. The exact mechanism of antibacterial activity between AgNPs tethered with acetaminophen and bacteria are debated here. Literature reports that the positive Ag ion is known to be antimicrobial property against different bacterial strains. Normally Ag must be an ionization state; in the ionized form, silver has inert state. When the Ag relates to humidity it delivers the silver ions. The silver ions can form complexes with nucleic acids and rupture the bacteria [28]

Synthesized of AgNPs exhibited a significant dose dependent inhibition of DPPH radical scavenging activity with a 50% inhibition (IC50) at a concentration of 259.06±1.3µg/mL. The IC50 value of vitamin C was 233.29±2.4µg/mL (Figure 3). In the synthesized AGNPs the ortho-dihydroxyl structure may be reason for the better antioxidant activity toward DPPH radicals. However, the ortho-hydroxyl phenoxyl radical and intramolecular hydrogen bond are involved in the process of oxidation for better stability. Previous reports also illustrated that the ortho-dihydroxyl would increase the rate of hydrogen atoms for radical transmission [29].

Table 1 represents larvicidal and pupicidal activity of AgNPs. The mortality and dose dependent response toxicity are improved when compared with the control group. The considerable larval and pupal mortality of Aedes aegypti synthesized AgNPs for all instars and pupae. Mortality increased as the concentration increased, for example, 35% mortality was noted in Ist instar larvae by the treatment at 2% of nanoparticle whereas it has been increased to 88% at 10% of treatment. Similarly, the same trend has been noted for all larval stages and pupae of Aedes aegypti at different concentrations of synthesized AgNPs treatment. The LC50 and LC90 values of mosquito larvicidal activity are represented in Table 1. The DMRT values and chi-square values are significant at 5% level.

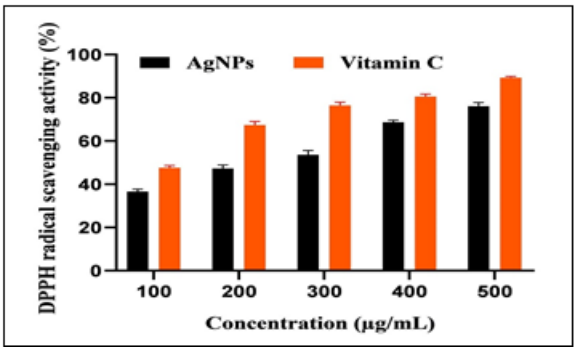

Figure 3: DPPH radical scavenging activity for particles

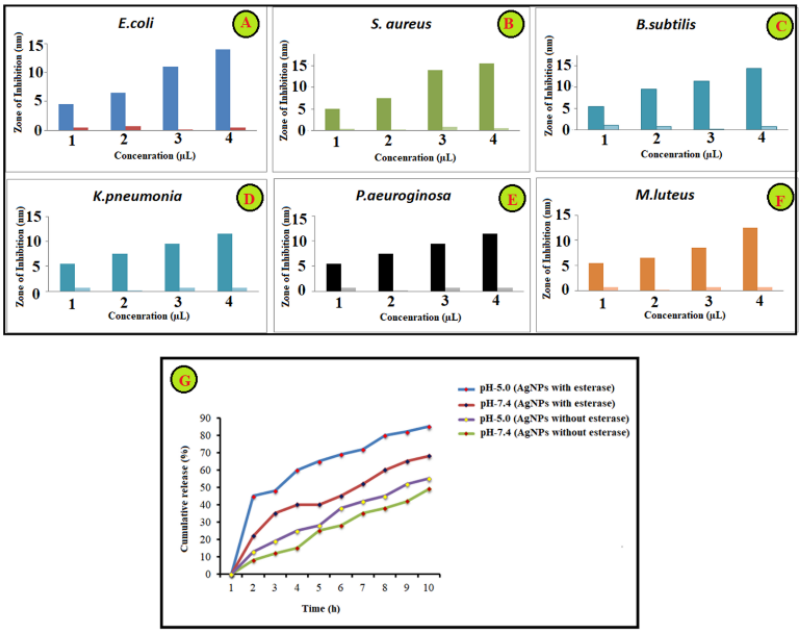

Figure 4: Antibacterial and drug release activity

Table 1: Larvicidal activity for synthesized nanoparticles

Larvae stages and pupae	% of larval and pupal mortality Concentration in ppm					LC50 (%)	LC90 (%)	Regression	Mean ± SE	95% Confidential Limit				Chi – Square (x^2) values
										LCL		UCL		
	20	40	60	80	100					LC_{50}	LC_{90}	LC_{50}	LC_{90}	
I	35	46	58	73	88	1.11	2.82	0.749(x) 0.832(y)	0.13	0.916	2.521	1.270	3.276	1.668
II	28	43	51	69	83	1.31	3.03	0.742(x) 0.975(y)	0.13	1.140	2.712	1.468	3.539	1.300
III	24	37	47	61	80	1.50	3.24	0.735(x) 1.106(y)	0.14	1.344	2.891	1.667	3.802	1.507
IV	21	27	40	56	75	1.73	3.44	0.749(x) 1.299(y)	0.14	1.579	3.065	1.911	4.033	1.998
Pupa	17	22	37	51	70	1.90	3.59	0.758(x) 1.444(y)	0.15	1.744	3.193	2.098	4.212	1.434

The in vitro drug release profile of acetaminophen from acetaminophen tethered AgNPs was performed by the dialysis bag method as shown in figure 3G. At both pH 5.0 and 7.4 without esterase, the AgNPs has 45% and 55% released the drug over the period of 10 h. In esterase, he AgNPs has 85% and 68 % drug release behavior at pH-5.0 and pH-7.4 respectively. Literatures report that the AgNPs were conjugated with different drugs which showed enhanced drug delivery through the electrostatic interaction [30]. From this study it is observed that the synthesized AgNPs has good drug releasing behavior at pH 5.0 with acid and esterase.

4. Conclusion

The current study concluded that AgNPs were synthesized from acetaminophen, with an SPR peak, smaller particle size, and a spherical morphology. The NPs demonstrated potential Larvicidal activity, suggesting that they might be exploited to generate new pesticides to control vectors. Controlling vectors helps to reduce vector-borne disease in the public health. For public use and successful non-toxic pesticides, more research on cytotoxicity effects on non-target species is essential.

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