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IN VITRO ANTIBACTERIAL, ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF CURCUMIN-ZINC OXIDE NANO-PARTICLES IN COMBINATION

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ABSTRACT

Nanoparticles have selective toxicity to bacteria but exhibit minimal effects on human cells. This study was carried out to investigate the antibacterial activity and potential hepatoprotective effect of curcumin-zinc oxide nanoparticles (1:1, w/w) on paracetamol-induced injury in rat hepatocytes. It was found that concentration 30µg was the highly effective against gram negative strains while concentration 10 µg was the most effective against gram positive strains. These strains were first examined by some antibiotic groups to detect the MDR ones. It was found that only *Streptococcus faecalis* was MDR. *Escherichia coli* and *Salmonella typhi* were the mostly affected strains they were scanned by TEM and it was found that nano curcumin affected the bacterial cell membrane and internal cell content. DNA fragmentation was done for *Salmonella typhi* and it revealed no change in DNA fragmentation. Also, incubation of hepatocytes with paracetamol resulted in increased formation of thiobarbaturic acid reactive substances (TBARS) with a parallel increase in lactate dehydrogenase (LDH) leakage as 1h following incubation. Time-dependent depletion of cellular reduces glutathione (GSH) was observed starting 2h following incubation with paracetamol. Incubation of hepatocytes with curcumin-ZnO nano-particles markedly protected against paracetamol-induced formation of TBARS, increase in LDH leakage and prevented GSH depletion. 20 µg is the most effective doses for curcumin-ZnO nano-particles. The results clearly suggest that curcumin-ZnO nano-particles in combination exerted a hepatoprotective effect against hepatotoxicity induced by paracetmol more pronounced than vitamin C.

KEY WORDS: Curcumin, zinc oxide, nanoparticles, antibacterial and hepatoprotective.

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INTRODUCTION

Nanoscale particles and molecules are a potential alternative for treatment of disease because they have unique biologic effects based on their structure and size, which differ from traditional small-molecule drugs[1]. Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin[2]. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and antimalarial activities. Because of the extended antimicrobial activity of curcumin and safety property even at high doses (12 g/day) assessed by clinical trials in human, it was used as a structural sample to design the new antimicrobial agents with modified and increased antimicrobial activities through the synthesis of various derivatives related to curcumin[3]. In the last few years, several pharmaceutical companies have obtained approval from the US Food and Drug Administration (FDA) for the development of nanotechnology-based drugs. The global market for medical nanotechnology is expected to reach more than $3 billion within the next few years[4]. Curcumin, a natural polyphenol found in the rhizomes of *Curcuma longa* (turmeric), exhibits anti-inflmmatory, antineoplastic, antioxidant, and chemo-preventive activity[5-9]. Several clinical trials dealing with cancers have addressed the pharmacokinetics, safety, and efficacy of curcumin in humans[7]. Despite extensive research and development, poor solubility of curcumin, due to its hydrophobic property and preferential interaction with lipid membranes, remains a major barrier in its bioavailability and clinical efficacy[3]. Zinc-oxide nanoparticles are amongst the most commonly utilized nano-materials in consumer products notably on account of their unique physicochemical properties[10,11]. It's also suggested that exposure to ZnO nano-particles led to a genotoxic potential mediated by lipid peroxidation and oxidative stress[12]. Vitamin C ability to act as a one- or two-electron reductant for a wide variety of oxidizing species has led to a great deal of interest in its role as a major antioxidant[13,14]. This role has been supported by many *in vitro* studies demonstrating the ability of vitamin C to scavenge oxidants and radical species including tocopherol, peroxy (LOO^o), singlet oxygen ($^1O_2$), thiyl (GS^o), O^{2-} and peroxynitrite radicals[15] and to protect the cells from damaging reactions of H_2O_2,nitrogen dioxide and X-irradiation[16,17]. Paracetamol is a widely used analgesic and antipyretic medication[18]. It has been available over the counter in the most countries since the late fifties[19]. Paracetamol causes acute hepatic necrosis in rats and other animal species[20]. The antioxidant functions of curcumin-zinc oxide nanoparticles have been characterized using a number of *in vivo* model systems based on scavenging of various reactive oxygen species or stable radicals[3,9,12]. But there are no reports of the effect of curcumin-ZnO nano-particles in combination on hepatotoxicity induced by paracetamol. This study was done to evaluate the antibacterial effect of curcumin-ZnO nano-particles on some pathogenic gram positive and gram negative bacteria with revealing the effect on bacterial DNA by DNA fragmentation assay and on bacterial cell wall by Transmission Electron Microscope (TEM). It was also aimed at investigating the hepatoprotective activity of curcumin-ZnO nano-particles by assessing the changes in GSH as well as LDH leakage and TBARS formation induced by paracetamol using isolated suspended rat hepatocytes. According to the available reports about the role of curcumin-ZnO nano-particles a potent antioxidant and paracetamol as a drug-induced cellular damage, this study was designed to evaluate the possible hepatoprotective effect, *in vitro,* of curcumin-ZnO nano-particles against hepatotoxicity induced by paracetamol and compare this effect by vitamin C as a standard antioxidant.

MATERIALS AND METHODS

1- Chemicals and Drugs

The antioxidant compound and chemical used in the present study were

1. Zinc oxide

2.5g Zinc-oxide nano- particles purchased from (Sigma, USA), Molecular formula ZnO, 99.5%: Molar mass, 81.408g/mol; Density, 5.606 g/cm^3 and insoluble in water.

2. Curcumin

10g Curcumin was purchased from (Sigma, USA). Curcumin has a melting point of 180°C; its molecular formula is ($C_{21}H_{20}O_6$) 99.8% pure and molecular weight 368.39.

Preparation of curcumin nanoparticles

Basic nano-curcumin is prepared by mixing the pure curcumin (8gm.) and sodium bicarbonate (32gm.) and grinding the mixture in the ball mail at 3500r.p.m for 8hrs that allow the solid reaction between the curcumin and bicarbonate so leading to disodium salt of curcumin nanoparticles was obtained. Sodium Bicarbonate ($NaHCO_3$) add to novel nano-composite: Approximately 0.5g of sodium bicarbonate ($NaHCO_3$) was added to adjust pH till alkaline pH 8.5 [21]. Curcumin-zinc oxide nanoparticles combination formula (1:1, w/w) was prepared at serial dilution; 5, 10, 15, 20, 25, 30 and 35µg suspended 1% Dimethyl sulfoxide (DMSO).

2-Bacterial strains

The following bacterial strains were used (ATCC, US) *Salmonella typhi* (ATCC: 14028), *Escherichia coli* (ATCC: 25922), *Klebsiella pneumoniae* (ATCC:13883), *Staphylococcus aureus* (ATCC: 25923), *Staphylococcus epidermidis* (ATCC: 12228), *Streptococcus faecalis* (ATCC:29212).They were sub cultured on Nutrient agar (Lab M, UK) and incubated aerobically at 37°C. Organisms were maintained in the laboratory on nutrient agar slopes at 4°C[22].

3-Antimicrobial susceptibility tests and detection of Multidrug resistant strains (MDR)

Antimicrobial susceptibility testing was done using the disk diffusion method, and results were interpreted using the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards (NCCLS) break point criteria [23]. Antimicrobial drugs included penicillin group (amoxicillin AML 30µg; glycopeptide group (vancomycin VA 30µg), aminoglycosides (amikacin AK 30µg), cephalosporin (cephradine CE 30µg) and Carbapemem (imipenem IPM 10µg). Multidrug resistant strains (MDR) were detected and defined as the non-susceptible strains to at least one agent in three or more antimicrobial categories [24].

4-Screening for curcumin-zinc oxide nano-particle antibacterial activity

Antibacterial activity was tested by agar well diffusion method on nutrient agar media using previously mentioned dilutions of curcumin-ZnO nano-particles prepared in 1% DMSO (5,10, 15, 20, 25, 30, 35µg).Wells were made using sterile borer and were filled with 0.45µl of each concentration. The antibacterial assay plates were incubated at 37ºC for 24h. The diameter of the zones of inhibition around each well was taken as a measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded [25].

5-DNA Fragmentation

The bacterial strain that is highly affected by curcumin-ZnO nano-particles was selected to detect the effect on the bacterial DNA using the DNA fragmentation essay

I-Samples preparation

Two tubes in duplicates (one contained 2ml nutrient broth, 20µl bacterial suspension and 50µl curcumin-ZnO nano-particles while the other tube is control without curcumin-ZnO nano-particles were incubated for 24h at 37°C.

II- DNA extraction

One milliliter was taken from each of the two tube cultures and was centrifuged at 10000 rpm, then the supernatant was discarded. The pellets were washed with 0.5ml of deionized distilled water (ddH_2O) and centrifuged again. The pellets were then resuspended in 100µl ddH_2O and subjected to heat block for 10 min at 98°C. Protein precipitation was done using 5.3M NaCl solution, centrifuged at 10000 rpm

for 10min. the supernatant of each tube was transferred to a new tube. DNA precipitation was done by adding double volume of isopropanol, centrifugation at 14000rpm for 20 min. DNA pellet was washed with 0.5ml of 70% ethanol, centrifuged at 14000 rpm for 10 min. the supernatant was discarded and the pellets were dried at room temperature and finally resuspended in 50µl ddH_2O[26].

III Agarose gel electrophoresis

Tris Acetate EDTA (TAE) electrophoresis buffer **(**50 X TAE stock) is prepared and stored at room temperature then diluted to 1X upon use in gel preparation or as running buffer. Gel loading dye (6x) was also prepared as (Bromophenol blue0.25% W/V, Xylene cyanol FF0.25%W/V and Glycerol 30%V/V)[26].

6-Transmission electron microscope (TEM) examination

Conventional TEM is frequently selected to visualize the ultrastructural damage on both cell wall and cytoplamatic membrane of entire microbes when fixed material can be used[27]. At ultrastructural level, a simple negative staining for TEM (JEM-1400 TEM, JEOL- Japan) of bacterial cells can report evidences on the mechanism of membrane disruption by antimicrobial proteins and peptides (AMPPs)[28]. The highly affected bacterial strain was scanned to show the effect of curcumin-ZnO nano-particles on the bacterial structure. Ultrathin sections obtained by conventional procedures, namely fixation with aldehydes, post-fixation with osmium tetraoxide, dehydratation and embedding in Epoxy resin, allow the observation of membrane and cytoplasmatic alterations. Treatment with AMPPs can induce several external and internal changes such as membrane bleb, ruffling or detachment, the presence of electrodense dots or fibers, hypodense cytoplasmic release and cell vacuolization[26]. The outer membrane detachment observed is generally related to the extremely high affinity of AMPPs to LPS, the main component of the gram-negative bacteria cell wall.

7-Experimental set up curcumin-zinc oxide nanoparticles on paracetamol-induced injury in isolated suspended rat hepatocytes Animals used

Female Wistar rats (about 3–4 months old, 250–300g) housed in temperature- and humidity-controlled room (24°C±2°C) with a 12-hour light/dark cycle and fed the standard laboratory diet and water ad-libitum were used. The guidelines issued by Animal Ethics Committee of October 6th University.

This experiment was carried out to examine the prophylactic potential of curcumin-ZnO nano-particles on paracetamol-induced injury in isolated suspended rat hepatocytes, *in-vitro*. The healthy rats were sacrificed by cervical decapitation and the healthy hepatocytes were performed using the collagenase perfusion method[30]. The obtained hepatocytes were suspended in Krebs-Henseleit buffer at a concentration of $5X10^6$ cells/ml. isolated suspended hepatocytes were then divided into 10 groups of rotating round-bottomed flasks, 4 in each and incubated with curcumin-ZnO nano-particles as the following

Group 1: DMSO and served for control negative.
Group 2: DMSO and served for control positive.
Group 3: Curcumin-Zinc oxide nanoparticles (5µg) suspended in DMSO.
Group 4: Curcumin-Zinc oxide nanoparticles (10µg) suspended in DMSO.
Group 5: Curcumin-Zinc oxide nanoparticles (15µg) suspended in DMSO.
Group 6: Curcumin-Zinc oxide nanoparticles (20µg) suspended in DMSO.
Group 7: Curcumin-Zinc oxide nanoparticles (25µg) suspended in DMSO.
Group 8: Curcumin-Zinc oxide nanoparticles (30µg) suspended in DMSO.
Group 9: Curcumin-Zinc oxide nanoparticles (35µg) suspended in DMSO.
Group 10: Vitamin C (3mM) in DMSO.

After a 30-min incubation period, the hepatocytes suspensions in groups 2, 3, 4, 5, 6, 7, 8, 9 and 10 were subjected to paracetamol (5mM). Samples of cell suspensions were

collected at different time intervals namely at zero time and 30min after incubation with curcumin-zinc oxide in combination and Vitamin C and before exposure to the paracetamol, then at 60, 120 and 180min. after addition of paracetamol. Each sample was divided into two parts (0.5ml each), one aliquot was used for the determination of TBARS level and the other one was centrifuged the clear supernatant was used for the determination of LDH activity, while the residue was for the estimation of GSH level[31].

8-Isolation and preparation of hepatocyte suspensions

The isolation of hepatocytes was performed using the collagenase perfusion method, as described by[32].

9-Biochemical investigation

Biochemical assays were carried out on isolated suspended hepatocytes as follows: LDH was determined according to the method of Buhl and Jackson[33], Thiobarbaturic acid reactive substances (TBARS)[32] as well as reduced glutathione (GSH)[34] was determined using commercially available kits (Asan and Young dong Pharmaceutical Co., Korea).

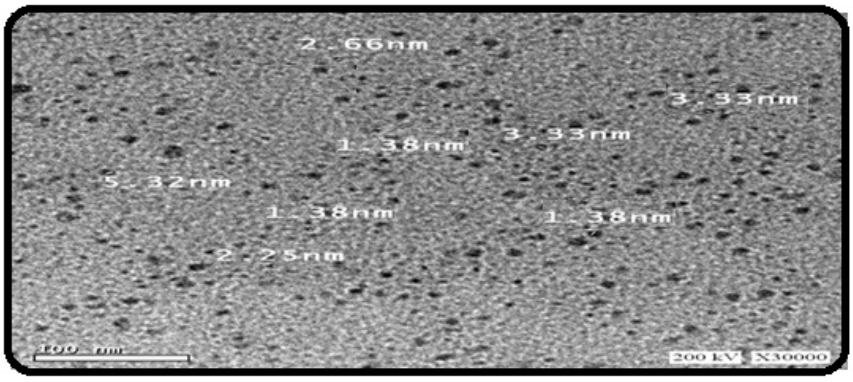
10-Statistical analysis

All data were expressed as mean of 6 replicates±SD. All analyses utilized SPSS,13.0 statistical package for Windows (SPSS,13.0 software, Inc.,[35]). A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value 0.05 was accepted as statistically significant. Control positive (group 2) was compared with control negative one (group 1). Curcumin-zinc oxide nanoparticles as well as vitamin C groups (3-10) were compared with positive control one (Group 2).

RESULTS

Curcumin-ZnO nanoparticles have bactericidal effects on both Gram-positive and Gram-negative bacteria. They even have antibacterial activity against spores that are resistant to high temperature and high pressure[36]. Determination of the size and shape curcumin nanoparticles by TEM (Transmission Electron Microscope).

Figure 1
Showed TEM image of the nano-curcumin; the dimension ranged from 1.38-3.33nm in diameter and had a spherical in shape



The antibacterial susceptibility test was done for the tested bacterial strain to investigate the MDR ones (table 1).

Table 1
Antibiogram of bacterial strains against different antibiotics and detection of (MDR)

Antibiotics	Mean diameter of inhibition zone(mm)				
	penicillin group	Carbapenem group	Cephalosporin group	Aminoglycosides group	Glycopeptide group
Bacterial strains	Amoxicillin (AML 30µg)	Impenem (IPM 10 µg)	Cephradine (CE 30 µg)	Amikacin (AK 30 µg)	Vancomycin (VA 30 µg)
Staphylococcus aureus	3.2 (S)	40(S)	15 (I)	28(S)	18(S)
Staphylococcus epidermidis	22(S)	45(S)	32(S)	40(S)	00(R)
Streptococcus faecalis	0.0 (R)	25 (S)	13 (I)	00 (R)	0.9 (R)
Klebsiellapneumonia	22(S)	18(S)	35(S)	25(S)	18(S)
Salmonella typhi	12(I)	38(S)	28(S)	22(S)	00 (R)
E.coli	23(S)	32(S)	30(S)	18(S)	00 (R)

- ***S: sensitive*** ***R: resistant*** ***I: intermediate***

The table illustrated that only *Streptococcus faecalis* was MDR strains. 77.7% of them were resistant to glycopeptides while 66.7% were susceptible to Penicillin group. The inhibitory effect of curcumin-ZnO nano-particles against the tested pathogenic gram positive and gram negative bacteria at different concentrations was done by the well diffusion method and illustrated in fig. (2, 3)

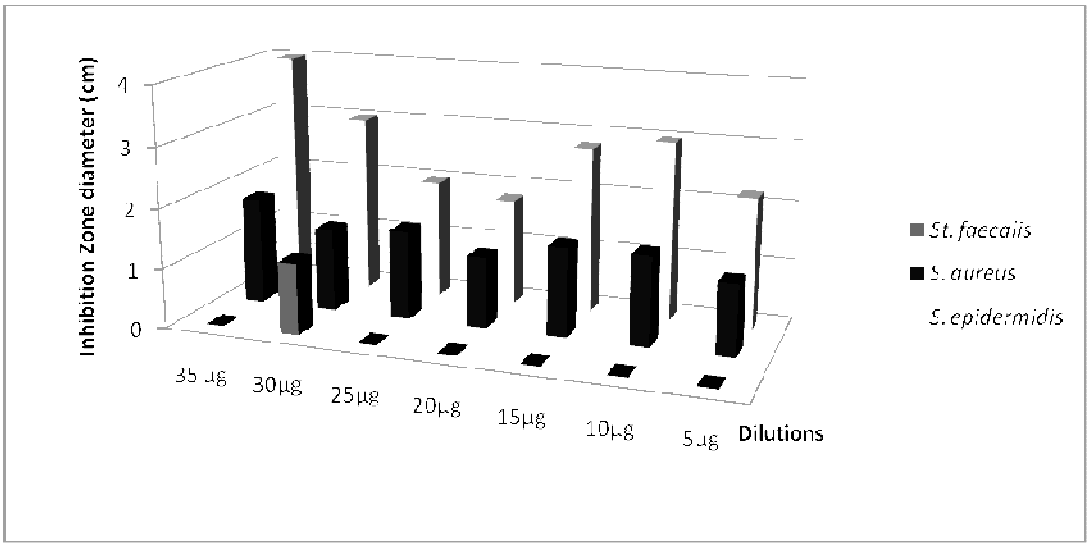


Figure 2
The inhibitory effect of curcumin-zinc oxide nanoparticle against the tested gram positive bacteria

Figure 2 represented that the highly affected bacterial strain was *Staphylococcus epidermidis* at all dilutions tested. *Streptococcus faecalis* was the least affected strain and the concentration 30µg was the highly effective concentration against the three strains.

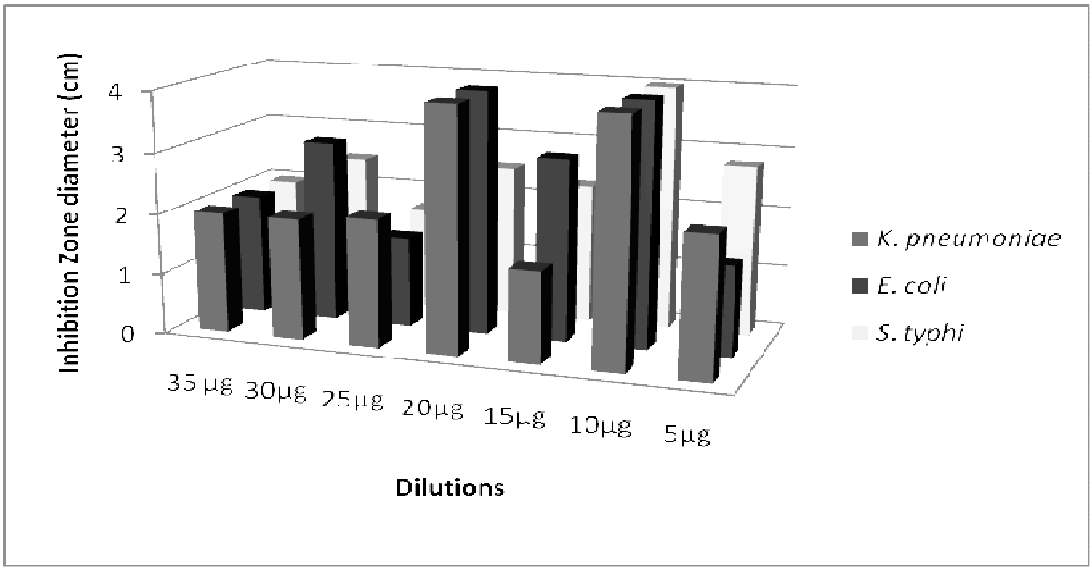


Figure 3
The inhibitory effect of curcumin-zinc oxide nanoparticle against some gram negative bacteria

Figure 3 showed that the tested gram negative bacteria affected at all dilutions but with different inhibition zone measures. The most effective concentration on the tested gram negative strains was 10µg. The figure also showed that the highly affected strains were *Escherichia coli* and *Salmonella typhi.* They were chosen to be scanned by TEM to demonstrate the effect of curcumin-ZnO nano-particles on them (fig. 4)

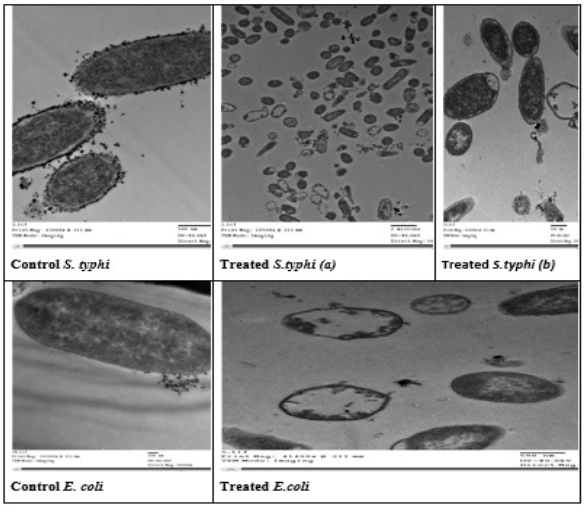


Figure 4
TEM for Salmonella typhi (control and treated (a and b) and E. coli (control and treated) with curcumin-zinc oxide nanoparticles

Figure 4 indicated highly effect of curcumin-ZnO nano-particles on internal bacterial content which disappeared in *S. typhi* treated (a) and treated *E. coli* while in treated *S. typhi* (b) there was changes in bacterial shape also which means that curcumin-ZnO nano-particles can affect both bacterial cell wall and protein content. DNA fragmentation assay was done

for *Salmonella typhi* (chosen as the highly affected strain) to investigate the effect of curcumin-ZnO nano-particles on fragmenting the bacterial DNA using agarose gel-electrophoresis (fig.5).

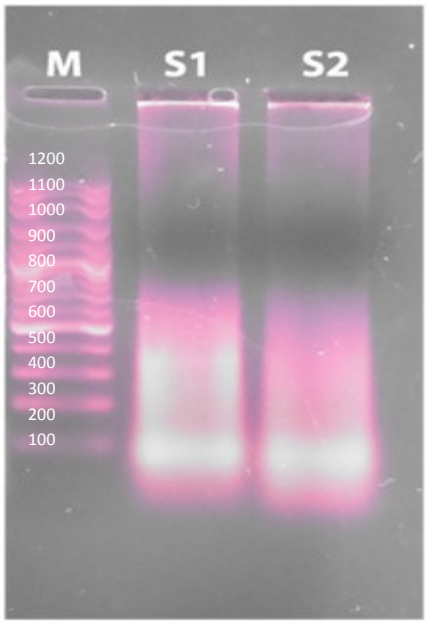


Figure 5
Agarose gel 1.5% showing DNA Fragmentation in control salmonella typhi (S1) and treated Salmonella typhi (S2) samples. M: 100 base pair molecular size marker

Figure 5 showed that there were no differences between the control and treated strain sample which means that curcumin-ZnO nano-particles could not change the bacterial DNA fragments. Tables 2 and 3 showed that the paracetamol (5mM) produced a marked increase in the leakage of LDH and TBARS within 60-180min. of incubation period. Also, elevation in leaked of LDH and TBARS to 2-4 folds as compared to the respective negative control ($p<0.01$). Preincubation of the hepatocytes with curcumin-ZnO nano-particles in combination (5-35µg) and vitamin C (3mM) offered a significant protection against paracetamol-induced LDH and TBARS leakage as early as 60min.as compared to the control positive group ($p<0.01$). The most effective doses for curcumin-ZnO nano-particles is 20µg. The results in table 4 illustrated that the paracetamol (5mM) produced a marked depletion of GSH within 120-180min of incubation period as compared to the respective negative control group ($p<0.01$). Prior incubation of hepatocytes with curcumin-ZnO nano-particles in combination showed a significant protection against paracetamol-induced GSH depletion detected as early as 120min after exposure to paracetamol and vitamin C (3mM) ($p<0.01$) as compared to the control positive group. 20µg of curcumin-ZnO nano-particles is more pronounced than other doses and vitamin C.

Table 2

Effects of different concentrations of Curcumin-Zinc oxide nanoparticles treatment on lactate dehydrogenase (LDH) leakage induced by paracetamol using isolated suspended rat hepatocytes

	% LDH leakage Time after addition of paracetamol (min)				
Groups	-30	0	60	120	180
Control negative(DMSO)	13.54± 1.44	14.00± 0.98	14.12± 1.25	13.98±1.88	13.56±2.00
Control positive(Paracetamol)	12.60± 2.14	17.13±2.08	30.76±2.25*	42.18± 3.75*	58.46±4.12*
Curcumin-Zinc oxide nanoparticles (5ug)	14.253± 1.40	16.11± 2.33	22.80± 1.79@	35.17± 3.00@	42.60± 3.59*
Curcumin-Zinc oxide nanoparticles (10ug)	14.64± 1.87	15.40±2.45	20.85± 2.70*	28.00±3.27*	35.68± 4.00*
Curcumin-Zinc oxide nanoparticles (15ug)	14.70± 1.90	16.00±2.30	18.60±2.54*	18.49±2.66*	22.54±1.50*
Curcumin-Zinc oxide nanoparticles (20ug)	14.30± 1.50	14.45±1.80	13.87± 1.65*	14.00±1.89*	14.50±1.35*
Curcumin-Zinc oxide nanoparticles (25ug)	13.80± 1.25	13.78± 1.67@	13.60± 1.84*	13.68±1.15*	14.35±2.88*
Curcumin-Zinc oxide nanoparticles (30ug)	14.07± 1.60	14.27±2.65@	13.50±2.70*	14.00± 3.00*	14.15±2.15*
Curcumin-Zinc oxide nanoparticles (35ug)	14.25± 1.86	14.35±1.24	13.80±3.57*	14.20±3.66*	14.25± 2.94*
Vitamin C(3mM)	13.66± 1.70	13.80@±2.00	17.20± 1.26*	17.60±2.65*	20.90±2.46*

@ Significantly different from normal group at P<0.05. * Significantly different from control group at P<0.01

Paracetamol (in DMSO) was added to the incubation media in all groups at a concentration of 5mM, except in the control negative group where only the vehicle DMSO was added. Paracetamol control group were compared with negative group. Experimental groups were compared with paracetamol positive control group.

Table 3

Effects of different concentrations of Curcumin-Zinc oxide nanoparticles treatment on Thiobarbituric acid reactive substances (TBARS) formation induced by paracetamol using isolated suspended rat hepatocytes

	TBARS (n mol/mL) Time after addition of paracetamol (min)				
Groups	-30	0	60	120	180
Control negative(DMSO)	0.055±0.005	0.057±0.003	0.060±0.004	0.054±0.006	0.050±0.002
Control positive(Paracetamol)	0.046±0.005	0.050±0.007	0.105±0.023*	0.180±0.022*	0.201± 0.04*
Curcumin-Zinc oxide nanoparticles (5ug)	0.055±0.004	0.057±0.004	0.083±0.025*	0.095± 0.007*	0.124±0.031*
Curcumin-Zinc oxide nanoparticles (10ug)	0.064±0.006	0.043±0.034	0.058±0.007*	0.085±0.018*	0.095±0.022*
Curcumin-Zinc oxide nanoparticles (15ug)	0.057±0.006	0.058±0.008	0.068±0.021*	0.073±0.006*	0.080±0.009*
Curcumin-Zinc oxide nanoparticles (20ug)	0.058±0.004	0.060±0.005@	0.052±0.009*	0.056±0.008*	0.053±0.005*
Curcumin-Zinc oxide nanoparticles (25ug)	0.063±0.004	0.069±0.006@	0.057±0.005*	0.0530.004*	0.052±0.008*
Curcumin-Zinc oxide nanoparticles (30ug)	0.052±0.008	0.060±0.004@	0.066± 0.007	0.066±0.008*	0.064±0.004*
Curcumin-Zinc oxide nanoparticles (35ug)	0.059±0.005	0.062±0.007	0.060±0.005*	0.063±0.008*	0.060±0.007*
Vitamin C (3mM)(3mM)	0.055±0.005	0.053±0.004	0.064±0.005*	0.077±0.006*	0.088±0.007*

@ Significantly different from normal group at P<0.05. * Significantly different from control group at P<0.01

Paracetamol (in DMSO) was added to the incubation media in all groups at a concentration of 5mM, except in the control negative group where only the vehicle DMSO was added. Paracetamol control group were compared with negative group. Experimental groups were compared with paracetamol positive control group.

Table 4

Effects of different concentrations of curcumin-zinc oxide nanoparticles treatment on cellular glutathione (GSH) leakage induced by paracetamol using isolated suspended rat hepatocytes

	GSH (nmol/10^6 cells) Time after addition of paracetamol (min)				
Groups	-30	0	60	120	180
Control negative(DMSO)	75.62± 5.11	73.20± 3.70	74.35± 2.60	74.90± 5.00	75.11± 6.15
Control positive(Paracetamol)	73.50± 4.22	61.25± 2.86	48.16± 3.87*	40.96±3.64*	33.68± 3.55*
Curcumin-Zinc oxide nanoparticles (5ug)	74.30± 2.60	66.15± 2.70	53.70± 2.52@	58.50± 4.08@	39.80±5.70@
Curcumin-Zinc oxide nanoparticles (10ug)	75.60± 6.19	69.80± 4.25	59.46± 5.33@	62.30± 6.80*	44.63± 3.22@
Curcumin-Zinc oxide nanoparticles (15ug)	73.20± 3.7	64.27± 4.22	61.80± 2.27@	64.58± 3.65*	69.11± 5.23*
Curcumin-Zinc oxide nanoparticles (20ug)	74.00± 6.40	69.43± 4.10	70.15± 4.68*	74.38± 4.09*	72.80± 6.43*
Curcumin-Zinc oxide nanoparticles (25ug)	76.49± 3.45	65.23± 3.89	68.45± 4.76*	70.25± 6.50*	68.72± 2.55*
Curcumin-Zinc oxide nanoparticles (30ug)	77.80± 6.75	66.18± 3.46	69.27± 5.22	71.00± 5.32*	71.86± 6.44*
Curcumin-Zinc oxide nanoparticles (35ug)	72.48± 6.23	68.90± 6.59	70.81± 3.47	72.30± 4.90*	69.35±5.308*
Vitamin C (3mM)	73.29± 4.37	65.10± 3.60	69.00± 6.33	71.10± 4.87*	70.13±2.88*

@ Significantly different from normal group at P<0.05.* Significantly different from control group at P<0.01

Paracetamol (in DMSO) was added to the incubation media in all groups at a concentration of 5mM, except in the control negative group where only the vehicle DMSO was added. Paracetamol control group were compared with negative group. Experimental groups were compared with paracetamol positive control group.

DISCUSSION

This study investigated the antibacterial effect of curcumin-ZnO nano-particles on six different gram positive and gram negative bacterial strains. This compound showed great effect on the tested bacterial strains except *Streptococcus faecalis.* The most effective concentration of curcumin-ZnO nano-particles was 10µg for gram negative bacteria (*Salmonella typhi, Escherichia coli*and*Kleb-siellapneumoniae*) while it was 30µg for the tested gram positive bacteria. Jones et al., [37] found that ZnO nanoparticles can adhere to the surface of the cell membrane, results in disturbance in its respiration as it interact with enzymes of the respiration chains of bacteria. In another study, Rai et al.,[38] showed that the antibacterial activity of nano-curcumin-ZnO against *S. aureus, B. subtilis, E. coli*, and *P.aeruginosa* exhibited a broad spectrum inhibitory effect against all microorganisms. MIC of nano-curcumin for *S.aureus, B. subtilis, E. coli*, and *P. aeruginosa* was 100, 75, 250, and 200µg/mL, respectively, compared to 150, 100, 300, and 250µg/mL for curcumin. A previous studies carried out by Bhawana et al.,[39] showed that curcumin-ZnO nano-particles inhibits bacterial surface protein sortase A and prevents cell adhesion to fibronectin, thereby acting as an antibacterial agent against *S. aureus*. He also found that, the mechanism through which curcumin nanoparticles are believed to manifest antibacterial properties is by anchoring to the cell wall of the bacterial cell, breaking it, then penetrating inside the cell, and disrupting the structure of cell organelles. Liu et al., [40] in his study revealed that the antimicrobial mechanism of ZnO nano-particles was therefore believed to destruct lipids and proteins on cell membrane, causing a leakage of intracellular contents as shown in TEM results This was in agreement to our result as the TEM showed that there was an effect on the cell wall and the internal content of both *E.coli* and *Salmonella typhi*. Plant-derived polyphenols minimize paracetamol-induced liver injury. Current therapy for fibrosis, such as ursodeoxycholic acid, does not prevent fibrosis[31]. Therefore, new strategies to prevent paracetamol-induced liver injury and fibrosis are needed. Previous studies suggest that oxidative stress occurs during fibrosis and likely

plays a role in paracetamol-induced liver injury[41]. Accordingly, antioxidant therapy represents a potential strategy to prevent liver injury and fibrosis. Previous studies show that antioxidants, including N-acetylcysteine, vitamin E, silymarin, and quercetin, decrease lipid peroxidation and partially ameliorate liver injury, but the effects of antioxidants on fibrosis remain controversial[40]. The results presented here show strong antioxidative effect *in vitro* on paracetamol-induced injury in isolated suspended rat hepatocytes. Depending on the test parameters (TBARS, LDH and GSH) the magnitude of the effects varied considerably between the different curcumin-ZnO nano-particles doses. The antioxidative effect increased in the order from 5 to 20µg. There is no difference in potency between the doses from 25 to 35µg with compared to 20µg at the levels of TBARS, LDH and GSH. The strong antioxidative influence of curcumin is in agreement with other reports[42-44]. In addition, the hepatoprotective and antioxidant effect of zinc oxide nanoparticles also reported[45,46]. Curcumin like other biologically active phytochemical[42,47,48]. Curcumin nanoparticles scavenge reactive oxygen species and free radicals by several proposed mechanism, including delocalization of electrons, formation of intramolecular hydrogen bonds[49] and rearrangement of their molecular structure[50,51.] Curcumin-zinc oxide nanoparticles in combination are capable of modulating the activity of enzymes and affecting the behavior of many cell systems and they possess significant antioxidant activities. The protective effect of vitamin C against hepatotoxicants observed in this study would be accepted. Vitamin C protection against paracetamol-induced GSH depletion was reported even in the presence of GSH depletors as diethyl maleate[51,52]. Thus although GSH normally functions to maintain vitamin C and other cellular components in the reduced states, vitamin C can serve as an essential antioxidant in the presence of sever GSH deficiency[53]. Interestingly, the protective effect of vitamin C demonstrated in the study of was only noted when it was administered 1h prior to paracetamol but not concomitantly with the hepatotoxicants, suggesting the superior prophylactic potential of this vitamin over its curative one[51].

CONCLUSION

In conclusion, the present study showed that a curcumin-ZnO nanoparticle in combination possesses antimicrobial activity against the tested gram positive and gram negative bacteria with destruction of bacterial cell wall and internal cell content. It also showed potent *in vitro* antioxidant activity against liver damage induced by paracetamol. Further studies are in progress to study the *in vivo* hepatoprotective effect of curcumin-ZnO nano-particles in combination against paracetamol-induced liver injury.

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