Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.0058

RESEARCH ARTICLE

N-Acetylcysteine Silver Nanoparticles as Antioxidant, Antimicrobial and Anti-cancerous against Sarcoma Male Rat Induced by 7,12 Dimethylbenz [a] Anthracene

Arwa H. AL-Saeed [1*], Alyaa S. Jasam[2], Ali B. Aldeewan[3], Muna H. AL-Saeed[4], Basil A. Abbas[5]

[1]Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq
[2,3,5]Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq
[4]Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

ARTICLE INFO

Received: May 4, 2024

Accepted: Jun 16, 2024

Keywords

Antioxidant
Anticancer
Antimicrobial
N-Acetylcysteine

*Corresponding Author:

ABSTRACT

The aim of this study is to investigate the biosynthesis of silver (Ag) nanoparticles using N-acetylcysteine (NAC) and effects of NAC-NPs on oxidative stress and sarcoma induced by 7,12 dimethylbenz[a]anthracene (DMBA) and antimicrobial. Silver nanoparticles are employed in many different fields, such as chemical and biological sensors, medicinal components, and catalysts. Studies on animals have demonstrated that chemicals are more carcinogenic to humans and animals. We examined sensitivity to a carcinogen in rats using a model of sarcomas-induction using a rarely performed subcutaneous injection of chemo-carcinogen. Male rats were injected with 150µg of 7,12-DMBA at 2 months,This caused 80% of the rats to develop sarcomas. In grossly observations Sarcomas form at the injection location. The result of NAC-AgNPs lead to significant ($p \leq 0.05$) weight gain and elevated body weight, CAT, SOD, GPx, MOP and TOS compared with DMBA group. The results showed significant decreas the MDA, ALP, AST, Calprotectin, IL-6, TNF-α, CRP, and TAS diameter and volume of tumors and significant ($p<0.05$) decreased the serum levels of serum p53 CA15.3 in therapeutic group. In the present investigation, silver nanoparticles were produced biologically by N-acetylcysteine (NAC) antioxidant, anticancer, anti-inflammatory, and their ability to combat harmful microbes with antibacterial properties We believe that these results could help clarify the mechanism underlying the formation of sarcomas in rats. NAC-AgNPs are one of the most popular applications for silver nanoparticles as antibacterial agents.

INTRODUCTION

Many characteristics are assigned to cancers, including their origins, their explosion, the defeat of segregation, and the attack of nearby tissues [1]. Chemotherapy, surgery, hormone therapy, and radiation are cancer therapies. These treatments have a number of side effects, such as toxicity to healthy cells and issues with drug resistance [2]. Natural goods were employed as an alternate strategy for cancer therapy in order to get around these issues [3]. Polycyclic aromatic hydrocarbons, or PAHs, are a common family of extremely lipophilic environmental organic contaminants that humans are exposed to on a regular basis [4]. Large amounts of PAHs are released into the environment, mostly as a result of human activity [5]. The biological effects, toxicity, mutagenicity, and carcinogenicity of PAHs are all negative [6,57]. Exposure to 7,12-dimethylbenz[a]anthracene (DMBA), which is a carcinogenic synthetic PAHs [7], underlies the emergence of numerous tumor types, such as those affecting the hematological system, skin, liver, lung, mammary gland, and

pancreas [8]. To become a fully carcinogenic substance, DMBA must undergo metabolic activation in the liver and extrahepatic tissues [9,53].

Noble metal nanoparticles (NPs) have been widely used in biomedical applications recently, including tissue engineering, medication transport, and diagnostics, because of their unique physicochemical and optoelectronic properties [10, 11, 12, 13]. Due to their excellent electrical conductivity, stability, optical property, and antimicrobial activity, silver nanoparticles (AgNPs), among other noble metal nanoparticles, have drawn a lot of interest in a range of applications, including nanoelectronic devices, sensors, imaging contrast agents, filters, and antimicrobial activity [14, 15]. AgNPs have expanded their use in cancer treatment as well. AgNPs have shown promise as potent anticancer agents [16, 17, 18, 19]. Strong anticancer efficacies mediated by apoptosis have been demonstrated in a range of cancer cells, including human cervical cancer [17,55], lung cancer [18], and breast cancer cells [19,56].

N-acetylcysteine (NAC), the acetylated precursor of L-cysteine, has long been utilized as a mucolytic drug and in the treatment of psychiatric illnesses, doxorubicin-induced cardiotoxicity, acetaminophen intoxication, and stable angina pectoris. Additionally, its level of toxicity is extremely low [8]. Because NAC is oxidized by different radicals and also functions as a nucleophile, it can eliminate protein disulfide bridges, operate as a free-radical capturer, and create metal chelation [20]. Moreover, NAC's anti-inflammatory and anti-apoptotic qualities are well recognized [21,54]. NAC works not just through these direct actions but also through raising GSH levels [19, 20].

MATERIALS AND METHODS

Animals. The University of Basrah's Health Check Explore Principled Team approved all experimental procedures in accordance with the Guide for the Care and Use of Animal Households. For these investigations, 32 Wistar rats weighing between 200 and 220 g were employed. Every rat was kept in a cage with a temperature of 23 ± 1 °C and a 12-hour light/dark cycle. During the period of adaptation, rats were fed conventional foodstuffs and given unrestricted access to tap water.

Experimental Design

Examining the prevalence of sarcomas in male rats given DMBA injections is the goal. Following a week-long period of adaptation, rats were divided into five groups (n = 8 per group), placed in the appropriate cages, and categorized as follows: group 1 (control): rats that were not treated and received a three-month injection of the vehicle; group 2: rats injected with 150μg/kg doses of DMBA dissolved in 0.05 ml sesame oil at the tumor site in the subcutis for two months and then left untreated for one month experienced a higher incidence of sarcomas. Group 3: The rats were treated with 150μg/kg doses of DMBA dissolved in 0.05 ml of sesame oil, which was injected into the subcutis for the incidence of sarcomas in rats for 2 months, and 4 mg/kg of NAC-AgNPs dissolved in 1 ml of normal saline for 1 month. Group 4: For a duration of one month, the rats were administered 4 mg/kg of NAC-AgNPs dissolved in 1 ml of normal saline. Two months later, the incidence of sarcomas was seen in rats injected with 150μg/kg doses of DMBA dissolved in 0.05 ml sesame oil subcutis injections. Group 5: The rats were treated with 4 mg/kg of NAC-AgNPs dissolved in 1 ml of normal saline for 3 months.

The tumor was tracked every week until the autopsy. The time between the day of injection and the day a nodular lesion first became palpably identifiable was referred to as the latent period. The rat was necropsied when the lesion grew to a diameter of more than 20 mm. All of the rats were euthanized at the necropsy.

Following a heart puncture under anesthesia (chloroform at 40 mg/kg body weight), tissues were removed from the animals, and blood samples were taken. Serum was obtained by centrifuging blood

samples for 10 minutes at 4000 rpm without the use of an anticoagulant. The serum was then kept at -20 °C until further biochemical examination.

measurement of pancreatic weight, body weight, and weight increase. Both at the beginning and the end of the trial, the animals were weighed [14].

measurement of the blood levels of MDA, SOD, GPx, catalase, TNF-α, and IL-6. After 60 days, the animals were slaughtered, and the serum concentrations of TNF-α (Abcam, Cambridge, UK) and IL-6 (RayBio, GA, USA) were measured using ELISA kits, following the guidelines provided by the manufacturer.

Antimicrobial Activity Assay

The testing microbes used in this study were previously characterized and obtained from the central lab in a veterinary college that were two Gram-positive bacteria (Staphylococcus aureus,Bacillus cereus, Escherichia coli, ,proteus and four fungal isolates Candida albicans, Aspergillus fimigatus, Aspergillus flavus, and Aspergillus terrus).

For microbial culture preparation, the testing bacteria were cultivated in nutrient broth at 37 oC for 18 h, whereas fungal isolates were cultivated in potato dextrose broth at 30 oC for 18 h. Then, the cell suspensions were adjusted to approximately 108 CFU/mL (equal to 0.5 McFarland standard).

Application of Discs

Following that, 10 mL of the chemical solutions with various concentrations that had been previously dissolved in DMSO (100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL) were soaked into the sterile paper discs (6 mm in diameter). [22].

Inoculum Preparation

After dipping a sterile cotton swab into the microbial suspension, the swab's excess fluid inoculum was removed by pushing it firmly against the tube's side wall above the fluid level. The sterile agar surface was then streaked with the inoculum. The agar's rim was swabbed as a last step after repeating this process twice more, spinning the plate at a temperature of roughly 60 °C each time to guarantee a uniform dispersion of inoculum.

Before applying the drug-impregnated discs, the plates were allowed to sit at room temperature for fifteen minutes to allow any excess surface moisture to be absorbed. After allowing the discs to air dry completely, they were placed on the infected medium's surface. Following inoculation, these plates were incubated for 24 hours at 37 degrees Celsius for bacteria and 30 degrees Celsius for yeasts and Aspergillus species. Every experiment was run through two duplicates. The information was gathered by using a ruler to measure the discs' surrounding zone of growth inhibition in millimeters.

Inoculation of Test Plates

After dipping a sterile cotton swab into the suspension, the swab's excess fluid inoculum was removed by pushing it firmly against the tube's side wall above the fluid level. The sterile agar surface was then streaked with the inoculum. The agar's rim was swabbed as a last step after repeating this process twice more, spinning the plate at a temperature of roughly 60 °C each time to guarantee a uniform dispersion of inoculum. Before applying the drug-impregnated discs, the plates were allowed to sit at room temperature for fifteen minutes to allow any excess surface moisture to be absorbed.

After tabulating the data as means and standard deviations (SD), analysis of variance (ANOVA) was used to compare the results, and post-hoc analysis (Tukey test) was performed. When the P-value ≤

0.05, a substantial difference was deemed to exist. Utilizing SPSS software (version 23), calculations were performed.

RESULTS

Induction of Sarcoma by 7,12 Dimethylbenz[a] Anthracene

The time between the day of injection and the day a nodular lesion first became palpably identifiable was referred to as the latent period. The rat was necropsied when the lesion grew to a diameter of more than 20 mm. At the necropsy, every rat was put to death. The hair near the tumor site was shaved, and in the event that the tumor invaded nearby organs, a large portion of the skin, muscles, and subcutaneous tissue were removed. Also examined were the cavities in the thorax and abdomen.

Therapeutic and Protective Effect of NAC-AgNPs on Body Weight and Body Weight Gain in Sarcoma Male Rats Induced by 7,12DMBA.

Table 1 of the current study shows a substantial ($P \leq 0.05$) reduction in the cancer male rat group's end body weight and body weight gain when compared to the control group (-ve). In contrast, there was a substantial ($P \leq 0.05$) increase in the final body weight and body weight gain in sarcoma patients treated with NAC-AgNPs (4 mg/kg dosage) and treated with NAC-AgNPs (4 mg/kg alone). The weight of the cancer male rats (+ve) treated with NAC-AgNPs (4 mg/kg dosage) increased significantly ($P \leq 0.05$) as compared to the control group (-ve),(Fig 2).

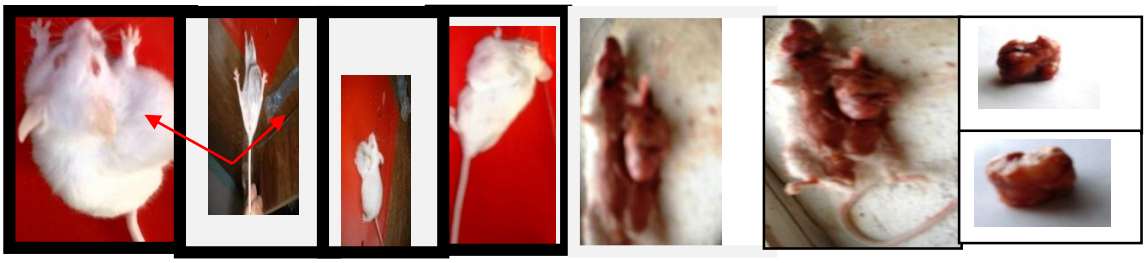


Fig.1:- Show tumor in Rat induction sarcoma

Table (1): -Therapeutic and Protective Effect of NAC-AgNPs on Body Weight and Body Weight Gain in Sarcoma Male Rats Induced by 7, 12DMBA. (Mean±SD) (*n*=8)

Parameters Treatments	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)
Control (-ve) Normal Saline(0.9% NaCl)	209.11± 0.47 NS	229.47± 2.75 A	20.6±0.33 a
Control (+ve) Induction sarcoma(7, 12DMBA)	211.58 ± 1.01 NS	170.67 ± 2.36 C	-40.94±0.11 d
Induction sarcoma(7, 12DMBA) + NAC-AgNPs(Therapeutic Group)	210.63 ± 3.01 NS	218.67 ± 8.01 B	8.04±0.26 c
NAC- AgNP+ Induction sarcoma (7, 12DMBA)(Protective Group)	213.43 ± 6.21 NS	226.17 ± 5.11 A	13.74±0.22 b
NAC- AgNP	207.73 ± 2.23 NS	232.23 ± 6.29 A	24.50±0.01 a

N=number of animals, Small letters denote differences between groups, P≤0.05 vs. control, NS=non-significant.

Therapeutic and Protective Effect of NAC-AgNPs on Antioxidant Stress in Serum SarcomaMale Rats Induced by 7, 12DMBA.

The impact of NAC-AgNPs on biochemical analysis in male sarcoma rats was noted in Table2 results. Significant (P<0.05) increases in ALP and ACP were observed, while significant (P≤0.05) decreases in serum sarcoma in male rats were suggested by the results of GPx and SOD levels. Additionally, the data revealed a significant (P≤0.05) drop in concentration as well as in ALP and ACP.

The most characteristic byproduct of lipid peroxidation is MDAn, and the body's level of lipid peroxidation can be determined by measuring its concentration. In comparison to the control group, the NAC-AgNPs-treated groups showed a substantial reduction in CAT and GST activity (P<0.05) and a significant elevation in MDA level (mg/dl) in all tissues investigated in this investigation. Pretreatment with Ag-NPs NAC revealed no significant difference (P>0.05) in any of the oxidative parameters in the control group, but all treated groups' serum showed a significant increase (P<0.05) in both CAT and GST activity and a significant decrease (P<0.05) in MDA level. These findings suggested that NAC-AgNPs protected against oxidative stress without impairing their antitumor activity.

Table (2): -Therapeutic and Protective Effect of NAC-AgNPs on Antioxidative Stress in Serum of Sarcoma Male Rats Induced by 7,12DMBA. (Mean±SD)

Parameters Treatment	GPx (mmol/L)	SOD U/dL	MDA mg/dl	CAT mg/dl	ALP U/L	ACP U/L
Control (-ve) Normal Saline(0.9% NaCl)	16.25 ± 0.86 a	91.3 ±1.29 a	103.67± 14.70 C	569.45±3.38 a	26.7±2.5 b	29.3±7.11 b
Control (+ve) Induction sarcoma(DMBA)	7.68 ± 0.15 b	56. 36±0.13 b	215.63±29.37 A	337.43±6.49 b	57.83±5.78 a	68.31±2.89 a
Induction sarcoma(DMBA) + Ag-NPsNAC	15.46 ± 0.37 a	89.45±0.28 a	149.93±5.51 B	506.67±9.27 a	27.66±3.69 b	31.47±3.86 b
Ag-NPsNAC+ Induction sarcoma (DMBA)	16.46 ± 0.11 a	90.05±0.33 a	123.13±5.91 B	546.17±9.27 a	26.55±3.69 b	28.26±3.74 b
Ag-NPsNAC	17.05±0.19 a	90.29±0.15 a	110.67±8.58 C	565.37±7.6 a	25.91±1.2 b	27.4±3.18 b

(*n*=8)

N=number of animals, A,B,C= differences between groups, P≤0.05 vs. control.

Therapeutic and Protective Effect of NAC-AgNPs on Calprotectin, IL-6, TNF-α ,CRP, MOP, TOS and TAS in Males Rats Induced by 7,12DMBA

Results in Table 3 showed how NAC-AgNPs affected inflammatory markers in male sarcoma rats. Results indicated a substantial (P<0.05) increase in Capl, IL-6, TNF-α, CRP, and TAS as compared to the control group and a significant (P≤0.05) drop in serum sarcoma male rats when it came to MPO and TOS levels.

Table 3. Effect Therapeutic and Protective of NAC-AgNPs on Calprotectin, IL-6, TNF-α و CRP, MPO, TOS and TAS in Sarcoma Males Rats Induced by 7,12DMBA (Mean±SD) (*n*=8)

Parameters Treatment	Calprotectin ng/ml	TNF-α ng/L	IL-6 Pg/ml	CRP Pg/ml	MPO ng/mg	TOS µmol/g	TAS Mmol/g
Control (-ve) Normal Saline(0.9% NaCl)	350.92 ± 10.01 b	120.12 ± 36.43 c	1.85±0.37 D	0.67 ± 0.011 c	1.12 ±0.29 b	0.27±0.01 A	0.11±0.02 c
Control (+ve) Induction sarcoma(7, 2DMBA)	3670.34 ± 39.09 a	200.60 ± 45.23 a	5.56±0.09 A	15.28 ±0.89 a	0.62 ±0.20 d	0.15±0.03 C	0.19±0.06 a
Induction sarcoma (7, 12DMBA) + NAC-AgNPs (Therapeutic Group)	765.41± 19.51 b	160.17 ± 14.21 b	2.26±0.27 C	7.77± 0.25 b	1.04±0.18 c	0.19±0.003 B	0.15±0.03 b
NAC- AgNP+ Induction sarcoma (7, 12DMBA) (Protective Group)	695.21± 14.71 b	150.18 ± 19.21 b	2.20±0.12 C	4.37± 0.44 b	1.01±0.16 c	0.16±0.001 B	0.13±0.01 b
NAC- AgNP	310.52 ± 14.36 b	140.11 ± 30.25 b	4.05±0.35 B	0.63 ± 0.012 c	1.64 ±0.32 a	0.30±0.02 A	0.12±0.05 c

N=number of animals, small letters denote differences between groups, P≤0.05 vs. control.

Therapeutic and Protective Effect of NAC-AgNPs on CA15.3 and tumor p53 in Serum Sarcoma Male Rats Induced by 7,12DMBA.

The effects of tumor p53 and CA15.3 in male rats with sarcoma are noted in Table 4. The findings revealed a significant (P<0.05) increase in tumor p53 and CA15.3 in male rats with serum sarcoma when compared to the control and other groups.

Table (4): -Therapeutic and Protective Effect of NAC-AgNPs on CA15.3 and Tumor p53 in Serum Sarcoma Male Rats Induced by 7,12DMBA (Mean±SD) (n=8)

Parameters Treatment	CA15.3 (U/ml)	Tumor p53 (pg/ml)
Control (-ve) Normal Saline(0.9% NaCl)	5000.70 ± 28.01 b	80.60 ±24.32 b
Control (+ve) Induction sarcoma(7, 2DMBA)	7000.96 ±70.8 a	140.32 ±32.19 a
Induction sarcoma (7, 12DMBA) + NAC- AgNPs (Therapeutic Group)	5500.37±52.90 b	120±17. 32 b
NAC- AgNP+ Induction sarcoma (7, 12DMBA) (Protective Group)	5100.37±52.90 b	94.23±10. 12 b
NAC- AgNP	4500.19±27.86 c	75.20±15.84 c

N=number of animals, a,b,c= differences between groups, P≤0.05 vs. control.

Therapeutic and Protective Effect of Ag-NPsNAC on Final Tumor Diameters and Tumor Volume in Male Rats Induced by 7,12-DMBA

The results in Table 5 show the effect of Ag-NPsNACon final tumor diameters and tumor volume in sarcoma male rats. The results showed a significant (P<0.05) increase in final tumor diameters and tumor volume in sarcoma male rats compared with the control group and other groups.While final tumor diameters and tumor volume in sarcoma male rats showed a significant (P<0.05) decrease in the group treated with Ag-NPsNAC.

Table 5: -Therapeutic and Protective Effect of Ag-NPsNAC on Diameter and Tumors volume in Male Rats Induced by DMBA (Mean±SD),(n=8)

Parameters Treatment	Tumor Diameter (mm)	Tumor Volume (cm3) rat-1
Control (-ve) Normal Saline(0.9% NaCl)	0.00 ± 0.00 c	0.00 ±0.0 c
Control (+ve) Induction sarcoma(7, 2DMBA)	40.61±1.705 a	25.35±2.01 a
Induction sarcoma (7, 12DMBA) + NAC- AgNPs (Therapeutic Group)	27.30±2.10 b	11.56±3.52 b
NAC- AgNP+ Induction sarcoma (7, 12 DMBA)(Protective Group)	0.00±0.00 c	0.09±0.00 b
NAC- AgNPs	0.00±0.0 c	0.00±0.00 c

N=number of animals, a,b,c= differences between groups, P≤0.05 vs. control.

Interpretation of Results

After 24-72 hours of incubation, the resulting zones of inhibition will be uniformly circular, and there will be a semi-confluent lawn of growth. The inhibitory zone diameters of the plates were measured and interpreted in accordance with the manufacturer's instructions (DHN PAN Kraków, Poland) after they were incubated at 37°C for 24 hours.

Effect of The NAC-AgNPs Against Bacterial Growth

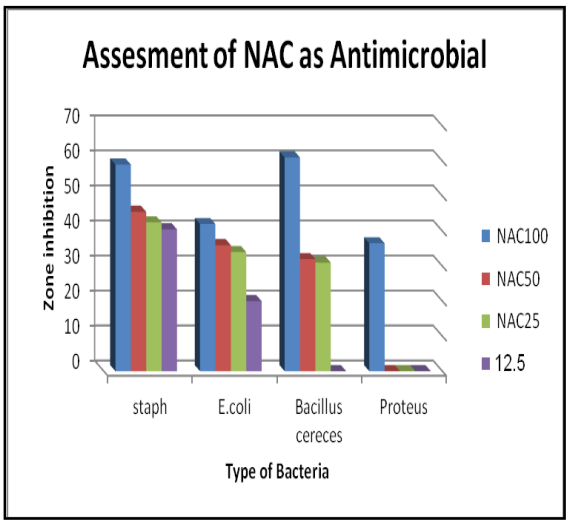
The results observed in Table 6 show that the NAC-AgNPs exhibited activity against gram-positive bacteria such as Staph. aureus and gram-negative bacteria such as *E. coli*. All the concentration studies showed a zone of inhibition against bacteria. The maximum antibacterial activity of NAC-AgNPs against Bacillus cereus, Staph. aureus, E. coli, and Proteus spp. was measured at 100 µg/mL of silver nanoparticles, with a zone of inhibition of 61±1.41, 59±1.41, 42.50±0.70, and 36.50±0.70 mm, respectively. Moreover, the zone of inhibition increased as the concentration of silver nanoparticles increased against all studied bacteria, and the zone of inhibition was greater than that of standard antibiotics (Fig. 2).

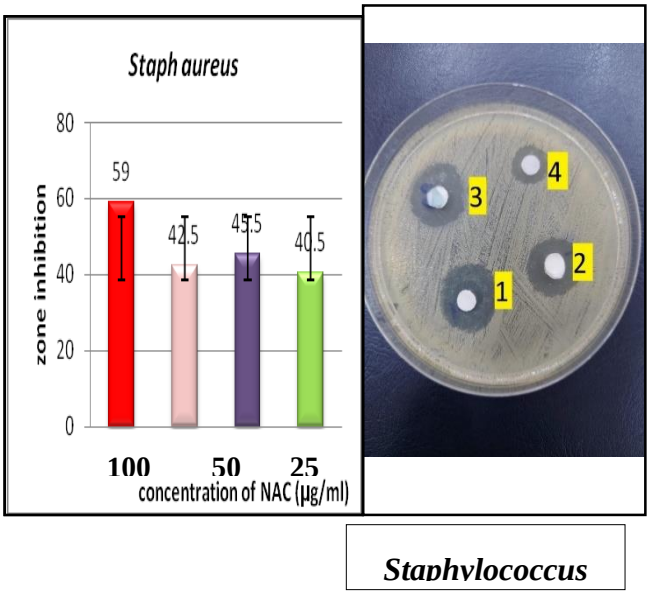
Table 6: Inhibition Zones in Diameter (mm) of NAC-AgNPs Against Bacterial Isolates

No.	Microbial isolates	Concentrations (µg/ml)			
		100	50	25	12.5
1	Staphylococcus aureus	59±1.41 aA	45.50±0.30 bA	42.50±0.70 bA	40.50± 0.20 bA

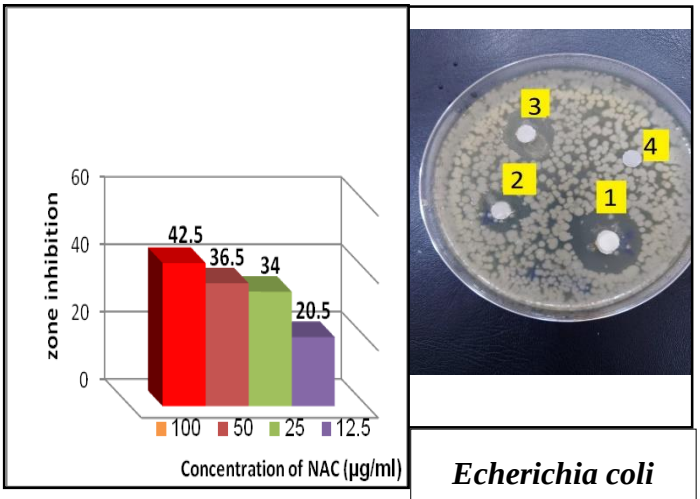
2	Bacillus cereus	61±1.41 aA	32±1.41 bC	31.50± 0.70 bB	0±0 cC
3	Echerichia coli	42.50±0.70 aC	36.50±0.70 bB	34±1.41 bB	20.50±0.70 cB
4	Proteus spp	36.50±0.70 aD	0±0 bD	0±0 bC	0±0 bC

Capital letter denote differences between groups, Small letters denote differences concentrations, P≤0.05 vs. control, NS=non-significant.

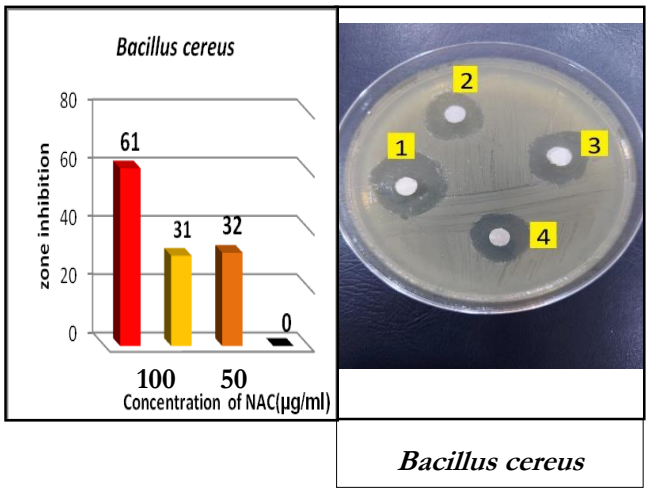




Staphylococcus



Echerichia coli



Bacillus cereus

Effect of Some Antibiotics Against Bacterial Isolates in this study

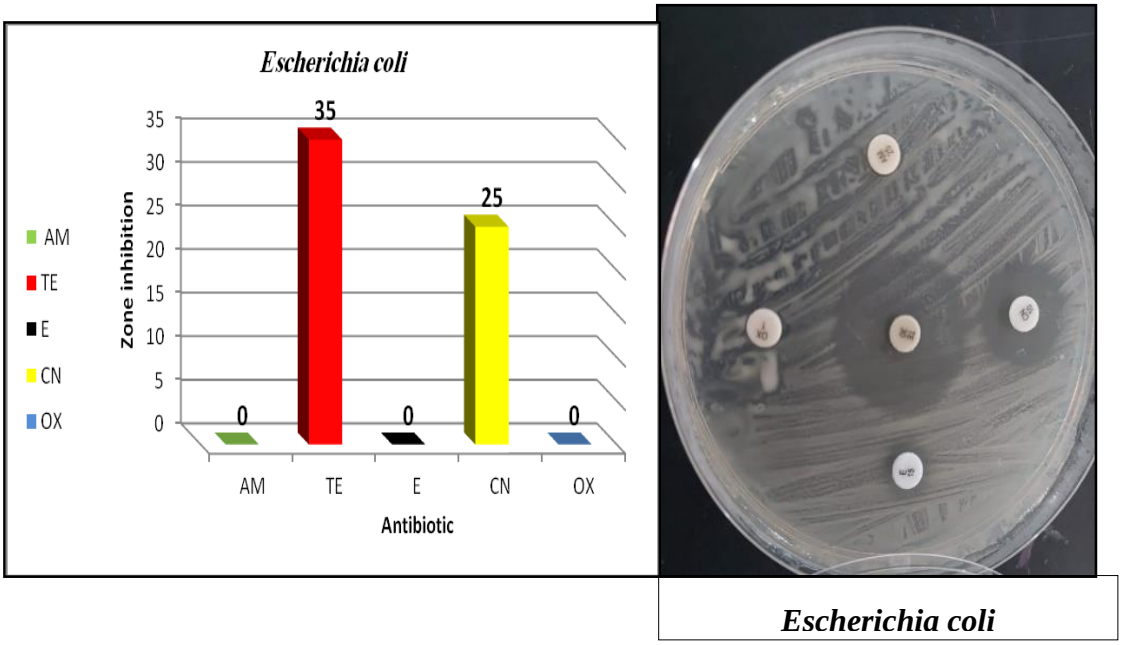
The results in Table 7 showed that standby antibiotics such as amoxicillin, erythromycin, and oxyacillin's exhibited zero zone inhibition against the gram-positive bacteria Staph. aureus and the gram-negative bacteria *E. coli.* But the stander antibiotics such as tetracyclines and gentamicin

exhibited zone inhibition of 35.27 ±0.32 and 38.12 ±0.66, 25.19 ±0.11 and ±270.16 against the gram-negative bacteria *E. coli* and the gram-positive bacteria Staph. aureus, respectively (Fig. 3).

Table 7: Effect of some antibiotics against bacterial isolates in this study

Bacterial species	AM	TE	E	CN	OX
Escherichia coli	0±0	35.27 ±0.32	0±0	25.19±0.11	0±0
Staphylococcus aureus	0±0	38.12±0.66	±00	±270.16	±00

AM=Amoxicillin TE=Tetracyclines E=Erthromycin CN=Gentamicin OX=Oxacillin's



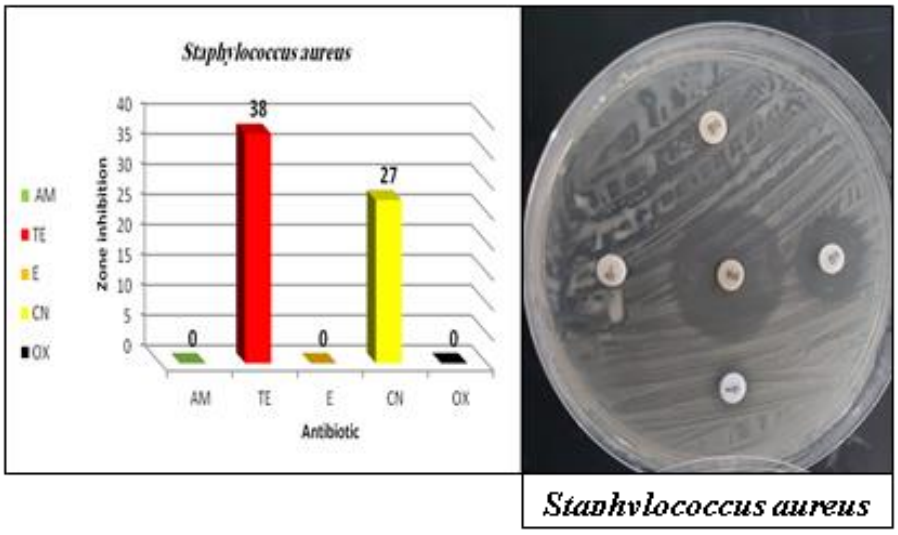


Fig. 3. Effect of some antibiotics against bacterial isolates.

Effect of NAC-AgNPs Against Fungal Isolates

The results observed in Table 8 show that the NAC-AgNPs exhibited activity against the fungi. A zone of inhibition was seen in the concentration studies. Concentration determined the zone of inhibition (Table 7). The maximum antifungal activity against Candida albicans, Aspergillus fumigates, and Aspergillus terrus with zones of inhibition were 50.50 ±0.70, 45.50 ±0.70, and 31.50 ±0.70 mm at 100 µg/mL of silver nanoparticles NAC-AgNPs, respectively; furthermore, there was an increase in the zone of inhibition with an increase in the fungi except Aspergillus flavus, which showed zero zone of inhibition in all studied concentrations of silver nanoparticles NAC-AgNPs against Aspergillus flavus (Fig 4).

Table (8) Inhibition Zones in Diameter (mm) of NAC-AgNPs Against Fungal Isolates

No.	Microbial isolates	Concentrations (µg/ml)			
		100	50	25	12.5
1	Aspergillus fumigatus	45.50±0.70 aC	38.50±0.70bB	0±0cC	0±0 cC
2	Candida albicans	50.50 ±0.70aB	30.50±0.70bC	31 ±1.41 bB	0±0 cC
3	Aspergillus terrus	31.50±0.70 aE	33.50±0.70aC	0±0 bC	0±0 bC
4	Aspergillus flavus	0±0F	0±0D	0±0 C	0±0 C

Capital letter denote differences between groups, Small letters denote differences concentrations, P≤0.05 vs. control, NS=non-significant.

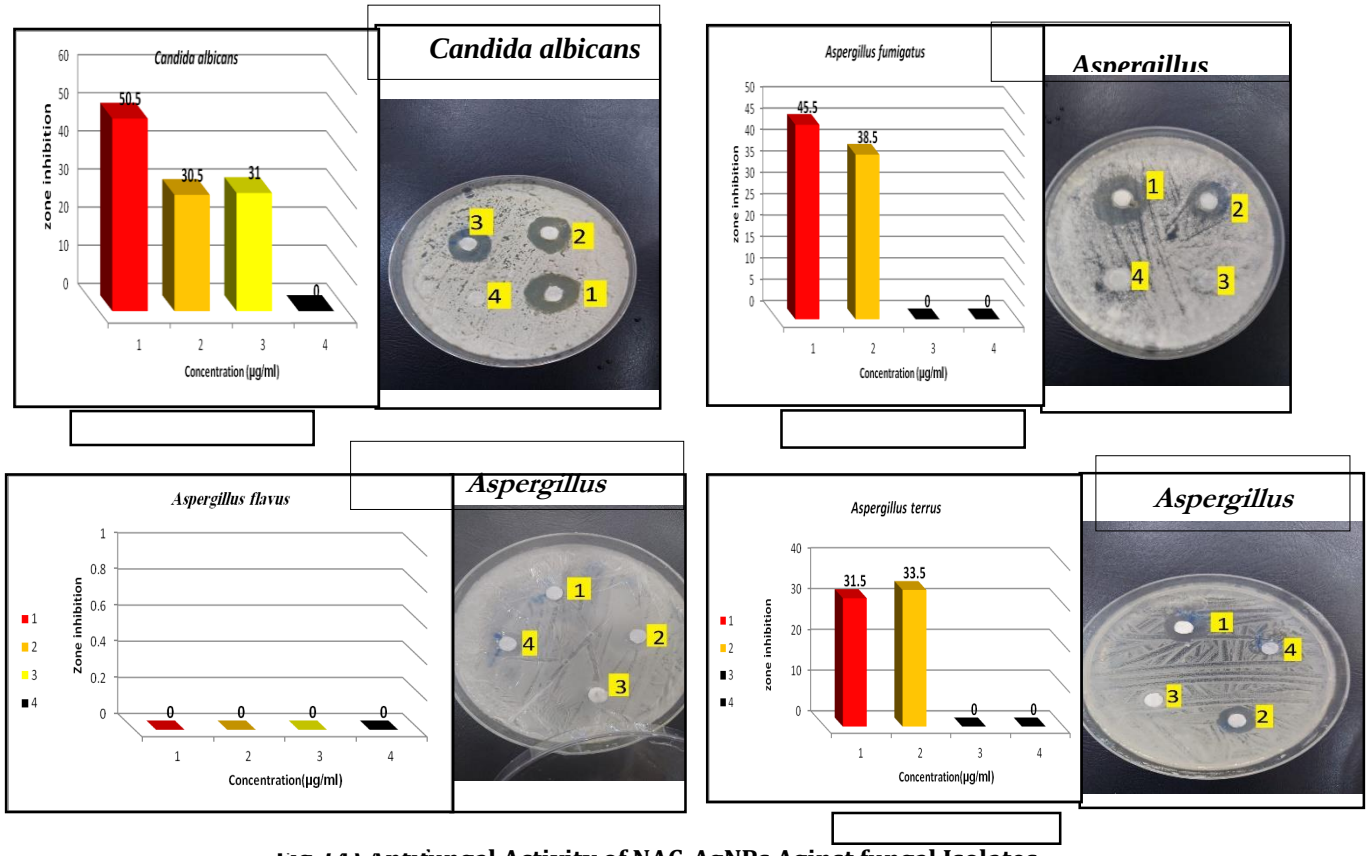


Fig. (4) Antifungal Activity of NAC-AgNPs Aginst fungal Isolates

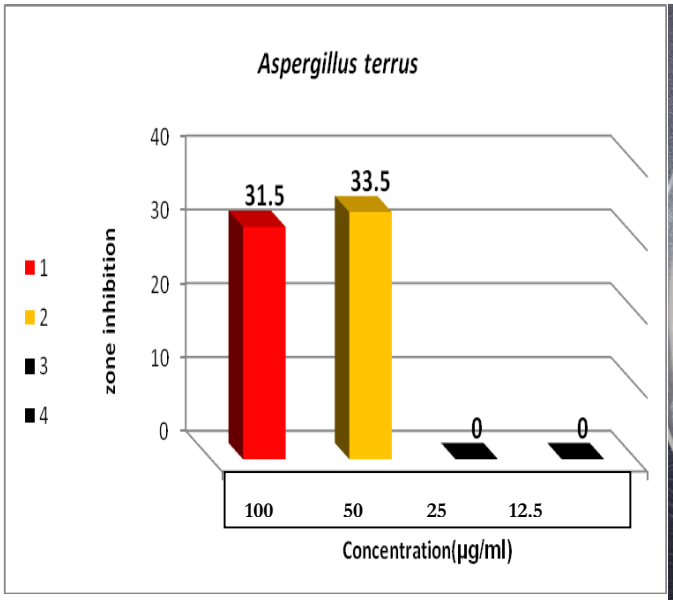
Effect of Some Antifungal Against Fungal Isolates in This Study

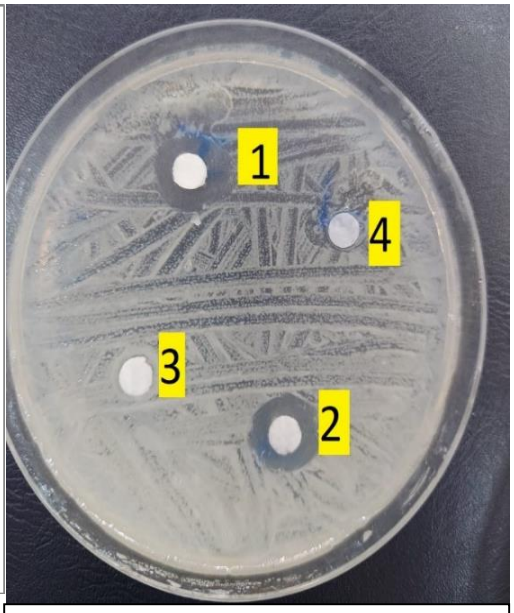
The results in Table 9 showed that stander antifungals such as posaconazole and econazole exhibited zone inhibitions of 20±1.06 and 22±0.16, 21±2.33 and 254.92, 18±2.28 and 20.2.81 against Candida albicans, Aspergillus flavus, and Aspergillus terreus, respectively (Fig 5).

Table 9: Effect of some antifungal against fungal isolates in this study

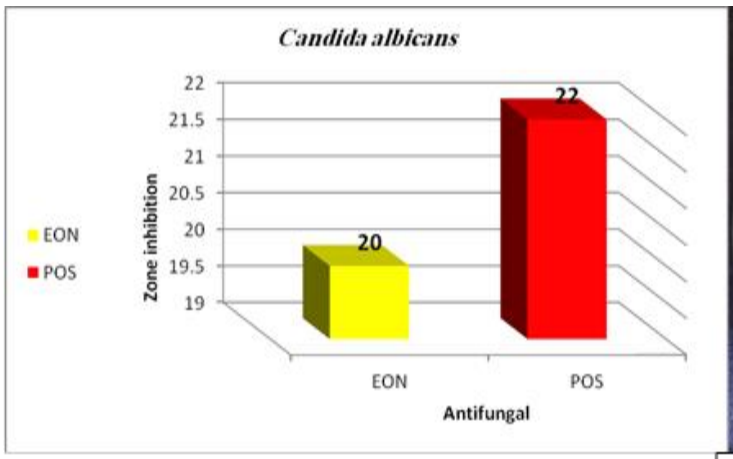
Fungal species	EON	POS
Candida albicans	20±1.06	22±0.16
Aspergillus flavus	21±2.33	±254.92
Aspergillus terreus	18±2.28	2.81±20

POS=Posaconazole EON=Econazole





Aspergillus terrus



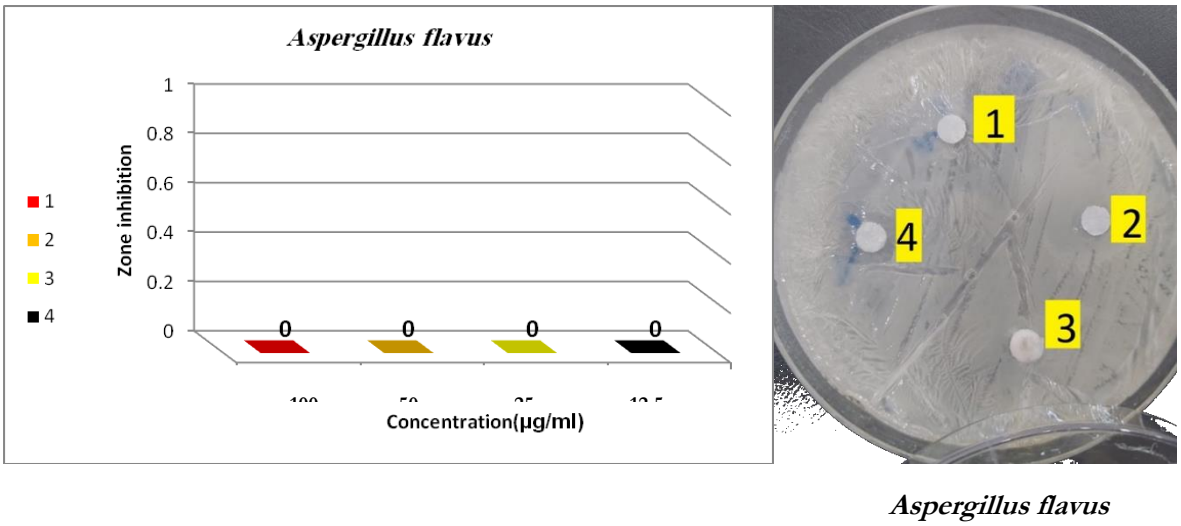


Fig. 5. Effect of some antifungal against fungal isolates

DISCUSSION

Rats with 7.12-DMBA-induced tumors are a well-known model that is frequently used to assess several substances as chemopreventive medications for human breast cancer [41]. The carcinogenic properties of DMBA are linked to its oxidative metabolism, which produces reactive metabolites. These metabolites bind covalently to nucleophilic sites on cellular macromolecules, triggering malignant reactions [42,58]. According to the available data, Ag-NPsNAC successfully decreased the tumor's dimensions and volume in rats that were tumor-bearing. We first looked into a few factors that contribute to the development of cell cancer in order to identify potential pathways for Ag-NPsNAC's anticancer impact. There are several ways to control the growth of cells. The nuclear protein PCNA, which is expressed in proliferating cells, is a hallmark of cell proliferation and is necessary for replication [43]. PCNA overexpression was identified. The proliferation of cells is controlled in several ways. The nuclear protein PCNA is a sign of cell proliferation and is required for replication in proliferating cells [43,51]. There have been reports of PCNA overexpression in a range of human malignancies, including DMBA-induced carcinoma. It is advised to assess the response of metastatic breast cancer to treatment and to monitor the serum marker CA15.3, which is frequently used for breast cancer diagnosis [45, 46]. Research has demonstrated that elevated blood levels of CA15.3 indicate a suboptimal response to immunotherapy (Martin et al., 2006). Rats were given oral Ag-NPsNAC therapy, as demonstrated.

Several investigations have shown that the status of the tumors CA15.3 and p53 has a significant role in determining how the cancers react to anti-neoplastic agents [47,50]. It has been claimed that 40% of all human cancers contain p53 mutations. Mutant p53 promotes cell growth, functions as an oncogene, and is no longer able to prevent tumor growth. By encouraging cell proliferation, overexpression of mutant p53 may worsen genetic instability [48]. According to our findings, the DMBA-treated group's serum levels of mutant p53 were noticeably higher than those of the control group. Infusion When comparing the DMBA group to the Ag-NPsNAC group, the mutant P53 serum levels were considerably lower. The current study's findings demonstrate that, at all employed concentrations, Ag-NPsNAC had an antibacterial action against all examined microorganisms, including filamentous fungus, candida, and Gram-positive and Gram-negative bacteria. Table 5 illustrates how the concentrations of Ag-NPsNAC increased the inhibitory zones against the investigated bacteria. When compared to other fungi, Aspergillus flavus is more resistant to NA Ag-NPsNAC C in all concentrations. Although the exact mechanism of Ag-NPsNAC's antibacterial activity against microbes is unknown, it is thought to target the cell's plasma membrane since NAC has an

amphipathic character that enables it to interact with the phospholipids in the membrane [39]. Another theory regarding the mechanism of action of NAC is that it causes microbial cells' membranes to become more permeable, which in turn alters the membrane and damages the cells [38]. In the current examination, we are looking into Ag-NPsNAC, which is regarded as a superior antibacterial.

Mixed-function oxidases must metabolically activate DMBA in order for it to have its carcinogenic and mutagenic effects. DMBA's 7-methyl group hydroxylation is an essential stage in the compound's carcinogenesis. As DMBA is further metabolized, a variety of metabolites with differing degrees of toxicity are formed. Among these, the carcinogenic byproduct of DMBA is trans-3,4-dihydrodiol-1,2-epoxide. When DMBA metabolic products are present in the body, they disrupt the equilibrium between ROS and antioxidants by producing excessive amounts of free radicals. In response, the body adjusts by modifying the activity of antioxidant enzymes to lessen the harmful consequences of elevated ROS. Numerous factors, including race, age, gender, pregnancy, lactation, muscle activity, geography, season, heat in the environment, maintenance, and diet, might have an impact on hematological and biochemical parameters. The effects of fluoride on liver histology and blood parameters are examined in this study. By destroying proteins and lipid membranes, oxidative chemicals like DMBA that are produced during mutagen metabolism may compromise essential cellular functions. For all cells, including bacteria, the oxidative state is crucial to their integrity and functionality [48, 49]. Here, we demonstrate how adding NAC to the Staphylococcus arueus biofilm in our in vitro biofilm system causes the bacteria's NAD+/NADH ratio to drop, indicating a significant degree of oxidative stress [47]. Following a 24-hour NAC administration, the NAD+/NADH ratio dramatically decreases as the NAC concentration rises. This phenomenon may be caused by either lowered or built-up NAD+ levels, which would raise the intracellular oxidative state. Reactive oxygen species (ROS) concentrations below a certain threshold are essential for bacterial growth suppression.

Multi-resistant bacteria are becoming more prevalent and are becoming a major global health concern due to their resistance to standard antimicrobial treatments. As a result, it is imperative to handle and control this problem using progressive alternative strategies, which have led to the discovery of fresh sources of antibacterial chemicals [37, 40].

In conclusion, we are presently conducting research to investigate the impacts of Ag-NPsNAC and its mode of action on bacteria that form biofilms, such as Staphylococcus. The purpose of these investigations is to ascertain whether Ag-NPsNAC has the same effect on other biofilm-forming bacteria as it does on P. aeruginosa. In the end, we will be able to produce complex biofilms in vitro to ascertain the most effective way to employ Ag-NPsNAC to kill bacteria and break down biofilm, as well as how to use it in conjunction with other medications using high-throughput screening, once we have a better understanding of how NAC affects the various biofilm-forming bacteria individually.

Multi-resistant bacteria are becoming more prevalent and are becoming a major global health concern due to their resistance to standard antimicrobial treatments. As a result, it is imperative to handle and control this problem using progressive alternative strategies, which have led to the discovery of fresh sources of antibacterial chemicals [37, 40].

REFERENCES

1-Tardiolo G., Bramanti P., Mazzon E.(2018). Overview on the effects of n-acetylcysteine in neurodegenerative diseases. *Molecules*. 23:3305.

2-Ooi, S.L.; Green, R. and Pak S.C. (2018). N-Acetylcysteine for the treatment of psychiatric disorders: A review of current evidence. *BioMed Res. Int.*8.

3-Aldini, G.; Altomare, A.; Baron, G.; Vistoli, G.; Carini, M.; Borsani, L. and Sergio, F. (2018). N-Acetylcysteine as an antioxidant and disulphide breaking agent: The reasons why. *Free Radic. Res*.52:751–762.

4- Dodd S., Dean O., Copolov D.L., Malhi G.S., Berk M. (2008). N-acetylcysteine for antioxidant therapy: Pharmacology and clinical utility. *Expert Opin. Biol. Ther.* 8:1955–1962.

5- Ezerina D., Takano Y., Hanaoka K., Urano Y., Dick T.P. (2018) N-Acetyl cysteine functions as a fast-acting antioxidant by triggering intracellular h2s and sulfane sulfur production. *Cell Chem. Biol.* 25:447–459.

6- Cuzzocrea, S.; Mazzon, E.; Costantino, G.; Serraino, I.; Dugo, L.; Calabrò, G.; Cucinotta, G.;De Sarro,A. and Caputi, A.P.(2000). Beneficial effects of n-acetylcysteine on ischaemic brain injury. *Br. J. Pharmacol.* 130:1219–1226.

7- Crupi R., Gugliandolo E., Siracusa R., Impellizzeri D., Cordaro M., Di Paola R., Britti D., Cuzzocrea S. N-Acetyl-L-cysteine reduces Leishmania amazonensis-induced inflammation in BALB/c mice. *BMC Vet. Res.* 2020; 16:13.

8-The chemistry and biological activities of N-acetylcysteine. *Biochimica et Biophysica Acta - General Subjects.* 2013. pp. 4117–4129.

9- Atkuri, K. R.Mantovani, J.J. Herzenberg, L.A. and Herzenberg, L.A. (2007).N-Acetylcysteine -a safe antidote for cysteine/glutathione deficiency. *Current Opinion in Pharmacology.* 7:355–359.

10-Zafarullah, M.; Li, W.Q.; Sylvester, J. and Ahmad, M. (2003). Molecular mechanisms of N-acetylcysteine actions. *Cellular and Molecular Life Sciences.* 60:6–20.

11-Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. (2016). Methods for in vitro evaluation antimicrobial activity: Areview. Journal of pharmaceutical Analysis. 6: 71- 79.

12-Sharma, M.; Kotwaal, A.; Thakuria, B.; Kakati, B.;Biswas ,D. (2016). Applicability of disk diffusion method for Antifungal sensitivity testing of Candida isolates in A clinical microbiology laboratory. International Journal of Health Sciences a nd research. 6 (6) : 90-93.

13-Lassa, H and Malinowski, E. (2007). Resistance of yeasts and algae isolated from cow mastitic milk to antimicrobial agents. *Bull Vet Inst Pulawy* 51: P 575-

14-Poncin S., Colin I.M., Decallonne B., Clinckspooor I., Many M.C., Denef J.F., Gérard A.C. (2010). N-acetylcysteine and 15 deoxy-{delta}12,14-prostaglandin J2 exert a protective effect against autoimmune thyroid destruction in vivo but not against interleukin-1{alpha}/interferon {gamma}-induced inhibitory effects in thyrocytes in vitro. *Am. J. Pathol*; 177:219–228.

15-De Andrade, K.Q.; Moura, F.A.; Dos Santos, J.M.; De Araújo, O.R.; De Farias Santos, J.C. and Goulart, M.O.F. (2015). Oxidative stress and inflammation in hepatic diseases: Therapeutic possibilities of N-Acetylcysteine. *Int. J. Mol. Sci.* 16:30269–30308.

16-Shahripour, R.B.; Harrigan, M.R. and Alexandrov, A.V. (2014). N-acetylcysteine (NAC) in neurological disorders: Mechanisms of action and therapeutic opportunities. *Brain Behav.* 4:108–122.

17-Rushworth G.F. and Megson I.L. (2014). Existing and potential therapeutic uses for N-acetylcysteine: The need for conversion to intracellular glutathione for antioxidant benefits. *Pharmacol. Ther.* 141:150–159.

18- Holdiness M.R. (1991). Clinical pharmacokinetics of N-Acetylcysteine. *Clin. Pharmacokinet.* ; 20:123–134.

19- Borgstrom, L.; Kagedal, B. and Paulsen, O. (1986). Pharmacokinetics of N-acetylcysteine in man. *Eur. J. Clin. Pharmacol.* ;31 :217–222.

20- Olsson, B.; Johansson, M.; Gabrielsson, J. and Bolme, P.(1988). Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. *Eur. J. Clin. Pharmacol.*34:77–82.

21-Jones, A.L.; Jarvie,D.R.; Simpson,D.; Hayes,P.C. and Prescott,L.F.(1997). Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver disease. *Aliment. Pharmacol. Ther.*11:787–791.

22- Lu, J. and Shenoy, K.A. (2017). Epithelial-to-pericyte transition in cancer. *Cancers*.9:1-13.
23-Kang, K.J. and Ahn, K.S. (2017) Anatomical resection of hepatocellular carcinoma: A critical review of the procedure and its benefits on survival. *World J Gastroenterol* 2017; 23(7): 1139-1156.
24 -Villeneuve, E.; Gosselin, S. (2017). N-Acetylcysteine. In: Brent, J.; Burkhart, K.; Dargan, P.; Hatten, B.; Megarbane, B.; Palmer, R.;White, J. editors. *Critical Care Toxicology.*
25-Prescott, L.F.; Donovan, J.W.; Jarvie, D.R. and Proudfoot A.T. (1989). The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. *Eur. J. Clin. Pharmacol.* ; 37:501–506.
26-Nolin, T.D.; Ouseph, R.; Himmelfarb, J.; McMenamin, M.E. and Ward, R.A. (2010). Multiple-dose pharmacokinetics and pharmacodynamics of n-acetylcysteine in patients with end-stage renal disease. *Clin. J. Am. Soc. Nephrol.*5:1588–1594.
27-Bonanomi, L. and Gazzaniga, A. Toxicological, pharmacokinetic and metabolic studies on acetylcysteine. *Eur. J. Respir. Dis. Suppl.* 1980; 111:45–51.
28- Rodenstein, D.; De Coster, A. and Gazzaniga A. (1978). Pharmacokinetics of oral acetylcysteine. *Clin. Pharmacokinet.* 3:247–254.
29- Papi A., Di Stefano A.F.D., Radicioni M. (2021). Pharmacokinetics and safety of single and multiple doses of oral n-acetylcysteine in healthy chinese and caucasian volunteers: An open-label, phase i clinical study. *Adv. Ther.* 38:468–478.
30-Toxicology Investigator Network A multicenter comparison of the safety of oral versus intravenous acetylcysteine for treatment of acetaminophen overdose. *Clin. Toxicol.* 2010; 48: 424–430.
31-Greene S.C., Noonan P.K., Sanabria C., Peacock W.F. Effervescent N-Acetylcysteine tablets versus oral solution n-acetylcysteine in fasting healthy adults: An open-label, randomized, single-dose, crossover, relative bioavailability study. *Curr. Ther. Res.* 2016; 83:1–7.
32-Yarema, M.; Chopra, P.; Sivilotti, M.L.A.; Johnson, D.; Nettel-Aguirre, A.; Bailey, B.; Victorino, C.; Gosselin, S.; Purssell, R. and Thompson, M. (2018). Anaphylactoid reactions to intravenous n-acetylcysteine during treatment for acetaminophen poisoning. *J. Med. Toxicol.* 14:120–127.
33-Homma S., Azuma A., Taniguiche H., Ogura T., Mochiuki Y., Sugiyama Y., Nakata K., Yoshimura K., Takeuchi M., Kudoh S. Efficacy of inhaled N-acetylcysteine monotherapy in patients with early stage idiopathic pulmonary fibrosis. *Respirology.* 2012; 17:467–477.
35-Kuzmenko N., Oleg I., Dmytro D., Roman I., Galyna G. The efficacy and safety of inhaled acetylcysteine in comparison with oral acetylcysteine in chronic obstructive pulmonary disease: A randomized single-center study. *Pol. Ann. Medicine.* 2020; 27:108–114.
36- Calverley P., Rogliani P., Papi A. Safety of N-Acetylcysteine at high doses in chronic respiratory diseases: A review. *Drug Saf.* 2021; 44:273–290.
37- Feng F., Zhang J., Wang Z., Wu Q., Zhou X. Efficacy and safety of N-acetylcysteine therapy for idiopathic pulmonary fibrosis: An updated systematic review and meta-analysis. *Exp. Ther. Med.* 2019; 18:802–816.
38- Liou G.G., Hsieh C.C., Lee Y.J., Li P.H., Tsai M.S., Li C.T., Wang S.H. N-Acetyl cysteine overdose inducing hepatic steatosis and systemic inflammation in both propacetamol-induced hepatotoxic and normal mice. *Antioxidants.* 2021; 10:442.
39- Mahmoudi G.A., Astaraki P., Mohtashami A.Z., Ahadi M. (2015). N-acetylcysteine overdose after acetaminophen poisoning. *Int. Med. Case Rep. J.* 8:65–69.
40- Abu-Mejdad, N.M. (2019). Isolation and molecular identification of yeasts, study their potential for producing killer toxins and evaluation of toxins activity against some bacteria and pathogenic fungi. Basrah: PhD thesis.: 1-249
41-Taylor, P.W. 2013. Alternative natural sources for a new generation of antibacterial agents Peter. Int. J. Antimicrob. Agents. 42 (3): 195-201

42-Al-Hussaini, R. and Mahasneh, A.M. (2011). Antibacterial and antifungal activity of ethanol extract of different parts of medicinal plants in Jordan. J. J. Pharmace. Sci., 4(1): 57 -69. (Cited by Boudkhili et al., 2012).

43-Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G. and Lightfoot, D.A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(42): 1-23.

44-Brooks, J.L. nda Jefferson, K.K. (2014). Phase Variation of Poly-N-Acetylglucosamine Expression in *Staphylococcus aureus*. *PLoS Pathog.* 10: e1004292.

45-Moormeier, D.E. and Bayles, K.W. (2017). *Staphylococcus aureus* biofilm: A complex developmental organism. *Mol. Microbiol.* 104:365–376.

46-Lee, S. F. and Davey, L. (2017). Disulfide bonds: a key modification in bacterial extracytoplasmic proteins," *Journal of Dental Research*, vol. 96, no. 13, pp. 1465–1473.

47-Fulda, S. Gorman, A. M. Hori, O. and Samali, A. (2010). Cellular stress responses: cell survival and cell death," *International Journal of Cell Biology*, vol. 2010, Article ID 214074, 23.

48- Guo, Y.; Li, Y.; Zhan, W.; Wood, T. K. and Wang, X. (2019). Resistance to oxidative stress by inner membrane protein ElaB is regulated by OxyR and RpoS," *Micro Biotech*, vol. 12; 2, 392–404.

49-de Graef, M. R.; Alexeeva, S.; Snoep, J. L. and Teixeira de Mattos, M. J. (1999). The steady-state internal redox state (NADH/NAD) reflects the external redox state and is correlated with catabolic adaptation in Escherichia coli. *J Bacterio.* vol. 181, no. 8, pp. 2351–2357.

50-Alneyadi, S., Abulibdeh, E., & Wardat, Y. (2023b). The impact of digital environment vs. traditional method on literacy skills; reading and writing of Emirati fourth graders. Sustainability, 15(4), 3418. https://doi.org/10.3390/su15043418

51-Alneyadi, S., Wardat, Y., Alshannag, Q., & Abu-Al-Aish, A. (2023a). The effect of using smart e-learning app on the academic achievement of eighth-grade students. Eurasia Journal of Mathematics, Science and Technology Education, 19(4), em2248. https://doi.org/10.29333/ejmste/13067

52-Wardat, Y., Tashtoush, M., AlAli, R., & Saleh, S. (2024). Artificial intelligence in education: Mathematics teachers' perspectives, practices and challenges. Iraqi Journal for Computer Science and Mathematics, 5(1), 60-77.

53-Zakariya, Y. F., & Wardat, Y. (2023). Job satisfaction of mathematics teachers: An empirical investigation to quantify the contributions of teacher self-efficacy and teacher motivation to teach. Mathematics Education Research Journal. https://doi.org/10.1007/s13394-023-00475-9

54-Jarrah, A. M., Wardat, Y., & Gningue, S. (2022b). Misconception on addition and subtraction of fractions in seventh-grade middle school students. Eurasia Journal of Mathematics, Science and Technology Education, 18(6), em2115. https://doi.org/10.29333/ejmste/12070

55-Tashtoush, M. A., AlAli, R., Wardat, Y., Alshraifin, N., & Toubat, H. (2023b). The impact of information and communication technologies (ICT)-based education on the mathematics academic enthusiasm. Journal of Educational and Social Research, 13(3), 284. https://doi.org/10.36941/jesr-2023-0077

56-Tashtoush, M. A., Wardat, Y., & Elsayed, A. M. (2023a). Mathematics distance learning and learning loss during COVID-19 pandemic: Teachers' perspectives. Journal of Higher Education Theory and Practice, 23(5). https://doi.org/10.33423/jhetp.v23i5.5933

57-Jam, F. A. (2019). Crypto currency–a new phenomenon in monetary circulation. Central Asian Journal of Social Sciences and Humanities, 4(1), 39-46.

58-Jam, F. A., Singh, S. K. G., Ng, B., & Aziz, N. (2016). Effects of Uncertainty Avoidance on Leadership Styles in Malaysian Culture, International Journal of Advance Business and Economics Research, 14(8), 7029-7045.