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RESEARCH ARTICLE

Polyphenolic Gum Arabic Extract with Antidiabetic, Antioxidant, Anticancer, and Antimicrobial Activities Enhances Blood Biochemistry, Heat Stress Genes, and Reproductive Hormones in Heat and Cisplatin Nephrotoxicity-Stressed Mice

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ABSTRACT

Gum Arabic (GA) extract, rich in polyphenols and phenolic acids, has shown promising effects in various biomedical applications. Acute renal injury (AKI) is a serious condition with high morbidity and mortality, while heat stress can exacerbate kidney damage. This study aimed to assess the antioxidant, anticancer, antimicrobial, and antidiabetic activities of GA extract, and to evaluate its protective role against heat stress and cisplatin-induced AKI in rats. A total of 210 mice were divided into seven groups (n=10 each), with three replicates: control, AKI, heat stress, low-dose GA (2%), high-dose of GA (10%), Cisplatin+high-dose of GA, and heat stress+high-dose of GA. Heat stress proteins (HSP70, HSP90), renal function markers (urea, creatinine), lipid profile (TC, TG, LDL, HDL) glucose and insulin level, sex hormones (testosterone, LH, FSH), oxidative stress parameters (MDA, CAT, SOD, GPx), inflammatory markers (TNF-α, IL-1β), and precancerous markers (BAX, Casp-3) were measured. The results indicated that GA extract demonstrated significant antioxidant, anticancer, antimicrobial, and antidiabetic activities, where scavenged 92% of DPPH free radicals, reduced 85% of breast cancer cell lines, and inhibited the growth of pathogenic bacteria and fungi in the MIC range of 0.5-2%. Furthermore, it inhibited the α-amylase and α-glucosidase by 78 and 85%, respectively. GA also ameliorated renal function markers and reduced oxidative stress by decreasing MDA, glucose, and insulin levels, while increasing CAT, SOD, GPx, and reproductive hormones levels. It downregulated pro-inflammatory cytokines and precancerous markers, increased HSP70 and HSP90 levels, and mitigated histopathological alterations in the kidneys of AKI rats. These findings suggest that the antioxidant and anti-inflammatory properties of GA contribute to its renoprotective effects. Thus, GA extract may offer a promising natural therapeutic approach for mitigating heat stress and managing cisplatin-induced AKI.

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INTRODUCTION

Heatstroke is a severe and potentially life-threatening condition marked by central nervous system disturbances such as delirium and seizures, along with a core body temperature exceeding 40°C (Sovioli *et al*., 2022). It is a form of multi-organ dysfunction caused by thermal injury to cells, systemic inflammation, and blood clotting abnormalities. This condition commonly occurs in hot environments, particularly in arid desert regions characterized by high temperature, low humidity, and intense ultraviolet radiations (Bukhari 2023).

Heatstroke and certain chemicals such as cisplatin, often cause acute kidney injury (AKI), which is marked by a sudden onset of damage to renal tissues (Masoud *et al*., 2024). AKI is further categorized into acute and chronic forms depending on its duration. Acute renal failure (ARF) involves a rapid decline in kidney function, including a reduced glomerular filtration rate, occurring within hours to days (Raza *et al*., 2020). This decline is accompanied by structural changes in the kidney structure, often leading to increased concentrations of urea and creatinine in the blood, electrolyte imbalance, and reduction in urine production (Yamamoto and Isaka, 2024). If left untreated, AKI can result in serious complications including chronic kidney disease, end-stage renal failure, and even death (Namazzi *et al*., 2022).

AKI can be caused by various factors, including exposure to substances that are toxic to the kidneys such as cisplatin (Kwiatkowska *et al*., 2021). Cisplatin is a chemotherapeutic drug commonly used to treat several cancers including ovarian, cervical, and breast cancer. However, it has a high risk of damaging the kidneys, which is one of its main side effects (Tchounwou *et al*., 2021). This kidney damage can reduce the effectiveness of cisplatin by causing AKI and disrupting the electrolyte balance in the body. For this reason, it is important to find a treatment that can protect the kidneys from the harmful effects of cisplatin without reducing its ability to fight against cancer (Fang *et al*., 2021).

Kidney injury caused by heatstroke or exposure to cisplatin is likely the result of several factors. These factors include reduced blood flow to the kidneys due to dehydration and low blood volume, direct heat damage to kidney tissues, muscle breakdown that releases myoglobin into the urine (a condition called rhabdomyolysis), and a widespread inflammatory response in the body. In severe cases of heatstroke, levels of inflammatory proteins like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) are significantly increased (Zhang *et al*., 2021). Histological examination often shows bleeding, blood blots, and cellular infiltration of white blood cells due to excessive kidney damage. Two important enzymes, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) play a major role in driving inflammation and oxidative stress, which further contribute to the damage (Ilari *et al*., 2020).

Heat shock proteins (HSPs) play a significant role in maintaining cellular survival by functioning as molecular chaperons and cytoprotective agents. These proteins enhance the ability of cells to withstand environmental and pathogenic stressors across various tissues and organs. Their protective mechanisms are primarily attributed to their capacity to assist in proper protein folding and facilitate the degradation of misfolded or damaged proteins, both under normal physiological conditions and during periods of stress. Research indicates that the level of HSPs decreases in conditions such as insulin resistance and aging, which may contribute to reduced cellular resilience. On the other hand, elevated production of HSPs has been associated with diminished oxidative stress, inhibition of inflammatory signaling pathways, and enhanced metabolic functions, particularly in tissues like skeletal muscles (Mayer, 2021; Kotler and Street, 2023).

Natural compounds have emerged as promising candidates for addressing AKI and heatstroke due to their multifaceted therapeutic properties. These compounds target the underlying pathological mechanism of disease including oxidative stress, inflammation, and cellular damage, that are central to the progression of these conditions. Specific natural agents, such as bioactive plant compounds, curcumin, and resveratrol exhibit strong antioxidant and anti-inflammatory properties and offer potential protection to renal tissues against injury (Abbas and Alkheraije, 2023; AlHoshani *et al*., 2023; El-Saadony *et al*., 2023a,b; Turan *et al*., 2023). Additionally, certain compounds that induce the expression of heat shock proteins may strengthen cellular resistance to stress. Other natural products have been shown to improve microcirculation and mitigate the severity of rhabdomyolysis, a common complication of heatstroke. Further investigation is required to fully establish their efficacy, safety, and mechanism of action because natural compounds represent a promising area of research for the development of innovative therapeutic approaches to combat AKI and heatstroke.

Grum Arabic (GA) is a natural product extracted from the sap of Acacia trees, particularly *Acacia Senegal* and *Acacia seyal*. This complex, branched chain hydrocolloid is primarily composed of arabinose and galactose sugars, along with proteins and minerals such as calcium, magnesium, and potassium. With a neutral to slightly acidic pH, GA has been utilized since ancient times, notably in Egypt, where it served as an adhesive in mummification and as a component in mineral paints for hieroglyphs. Today GA is widely used across various domains including the food and pharmaceutical industries. Medicinally it is employed to address respiratory and gastrointestinal issues, support liver and kidney health, and manage skin and inflammatory conditions. Additionally, GA has shown its therapeutic potential in treating chronic hepatitis and combating various viral infections (Idriss *et al*., 2023).

Research has demonstrated that GA extract possesses strong antibacterial properties, particularly against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA). It achieves this by reducing lipase activity and suppressing the expression of virulence genes (Lakshmi *et al*., 2020). Additionally, GA has the potential to increase burn wound healing due to its anti-inflammatory, antimicrobial, and antioxidant effects. It promotes wound contraction and shrinkage, reduces pain and discomfort, stimulates epithelialization, and increases angiogenesis, all of which contribute to faster recovery in burn wound healing (Sharma *et al*., 2020).

However, no available studies showed the mitigating effect of GA extract on heat and cisplatin stressors in mice. Therefore, in this study, the active compounds in GA extract were detected using Gas chromatography-mass spectroscopy (GC-MS). Attempts were also made to examine its antioxidant, anticancer, and antimicrobial activity, elucidating its effects aqueous on the heat and cisplatin stressors through monitoring the biochemical, molecular and histological changes in the kidney of stressed mice.

MATERIALS AND METHODS

Preparation of gum Arabic extract: Aqueous gum Arabic (GA) powder extract was prepared following the Todorović *et al.* (2022) method with some modifications. Briefly, 20g of GA powder was mixed with 180mL of deionized water and homogenized. Using an orbital shaker, the mixture was then agitated at 200rpm for 1h at room temperature. The extraction was repeated twice; the filtrates were combined and concentrated using a rotary evaporator at 55°C under reduced pressure.

Determination of active compounds profile of GA extract: The GC-MS analysis was conducted on an aqueous extract of GA using a Shimadzu QP2010PLUS GC-MS system. The system employed a capillary column and utilized split-less injections with a purification time of 0.1min. Helium was the carrier gas at a 1mL/minute flow rate. The column temperature was programmed as follows: 50°C for 3min, followed by a ramp of 5°C/minute to 80°C, and then 10°C/minute to 340°C. The inlet and detector temperatures were set at 250 and 340°C, respectively, with a solvent delay of 4 minutes. Peak identification was achieved by comparing the obtained mass spectra with entries in the National Institute of Standards and Technology library (NIST 08 and NIST 08S) and referencing previously published data (Edeoga *et al.*, 2005).

Determination of biological activities of GA extract

Antioxidant activity: The antioxidant activity of GA was evaluated by mixing 1.0mL of GA (2, 4, 6, 8, and 10%) with 3mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH); the GA concentrations were prepared by water dilution of pure GA powder, the mixture was maintained for 60min. Optical density (OD) the developed color was measured at 517nm using a colorimeter (Alowaiesh *et al.*, 2023). Then, the following formula was used to determine the antioxidant activity of the GA solution:

$$\% \text{ Antioxidant activity} = \frac{\text{OD of DPPH} - \text{OD of sample}}{\text{OD of DPPH}} \text{x } 100$$

Antibacterial and antifungal activity: The bactericidal activity of GA was evaluated using a disc diffusion assay (El-Saadony *et al.*, 2021a). The plate count agar (PCA) plates were inoculated with 100µL of *Bacillus cereus*, *Streptococcus aereus*, *Escherichia coli*, and *Klebsiella pneumoniae* cultures. Discs (8mm) saturated with varying concentrations of GA were placed on the PCA surface. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibitory zones (IZDs) were measured in millimeters as outlined in previous works (El-Saadony *et al*., 2021a). The minimum inhibitory concentration (MIC) of the GA extract was determined using the method described by Saad *et al*. (2021a); Almuhayawi *et al.* (2023).

The antifungal activity of GA at concentrations ranging from 2-10% was evaluated against six pathogenic fungi including *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Alternaria alternate*, *Pythium aphanidermatum*, and *Podosphaera xanthii.* These fungi were isolated from spoiled feed and identified through genetic analysis using 16S rRNA gene sequencing. Fungal suspensions were inoculated onto potato dextrose agar (PDA) plates. Sterile paper discs impregnated with different GA concentrations were placed on the inoculated plates and incubated at 25°C for 7 days. After incubation, the inhibitory zone diameters were measured in millimeters, following the procedure described by El-Saadony *et al*. (2021b).

For SEM analysis, fungal and bacterial samples were prepared by centrifuging the bacterial broth and washing the resulting pellet or hyphae three times with phosphate-buffered saline. The samples were then fixed with 0.25% glutaraldehyde in sodium phosphate buffer (pH 7.2) for 30 minutes at room temperature. After fixation, the samples were incubated overnight and washed three times with sodium phosphate buffer. The resulting pellet was collected by centrifugation and dehydrated through a series of increasing ethanol concentrations (30, 50, 70, 80, 90, and 100%), with 10-minute incubation for each step and a final 1-hour incubation in 100% ethanol. Finally, adhesive tape was applied to the dehydrated microbial sample and placed on an SEM stub for analysis (Ammar, 2017).

Anticancer activity: Breast cancer cell lines obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA) were grown as a single layer. These cells were then enzymatically detached using trypsin and subsequently counted. The cell count was adjusted to a concentration of 100,000/mL using fetal bovine serum (FBS)-enriched Dulbecco's Modified Eagle Medium (DMEM). Subsequently, 100µL of this diluted cell suspension, containing approximately 10,000 cells, was carefully introduced into each well of a 96-well microtiter plate. The plate was then placed in a CO_2 incubator maintained at 37°C for 24h. The culture medium was then replaced with fresh medium supplemented with varying concentrations of FBS and GA (2-10%), followed by an additional 48h incubation at 37°C. Cells were detached from their culture surface using a trypsin-EDTA solution. Trypan blue staining was then used to differentiate between live and dead cells. The number of viable cells was counted. The results were recorded as % inhibition of cell viability (Motafeghi *et al.*, 2024).

Antidiabetic activity: The GA inhibition activity of α-glucosidase was evaluated calorimetrically as follows: GA extract (2-10% concentrations) was incubated with α-glucosidase at pH 6.9 and 37°C for 10 min. Then, 50µL of a 1mM p-NP-α-D-glucopyranoside solution (in PB) was added, the reaction was stopped after 10min, the OD was

recorded at 405nm and then applied in the following equation (Carneiro *et al.*, 2022):

$$\%\ \alpha - glucosidase\ inhibition\ activity = \frac{OD\ control - OD\ sample}{OD\ control} x100$$

The α-Amylase inhibitory activity was assessed following the method of Nair *et al.* (2013). Briefly, 50µL of GA concentrations were mixed with 1mg/mL acarbose and 500µL of α-amylase in phosphate buffer, pH 6.8 and incubated for 10min at room temperature. Then, 500µL of soluble starch was added and incubated as the previous step. Next, 1mL of 96mM 3,5-dinitro salicylic acid was added, and the mixture was heated for 5 minutes, then cooled to 25°C. The OD was read at 540nm and applied to the following equation:

$$\%\ \alpha - amylase\ inhibition\ activity = \frac{OD\ control - OD\ sample}{OD\ control} x100$$

Experimental layout: A total of 210 male mice, aged 6 days and weighing 30-42g, were obtained from the local mice breeding facility. These mice were allowed to acclimatize to the laboratory environment for two weeks and housed in plastic cages under controlled conditions. The housing environment was a 12-hour light-dark cycle, with a 23.2°C temperature and 40-60% relative humidity. The mice had free access to water and a standard basal diet (NRC, 1994) throughout the experimental period. The experimental design comprised seven groups with 10 mice per group and three replicates, as follows: NC, negative control, offered basal diet for four weeks; PC1, positive control, offered basal diet and challenged with a single intraperitoneal (i.p.) injection of 4.5mg/kg cisplatin (dissolved in 0.9% NaCl) for two consecutive days; PC2, positive control, offered basal diet under heat stress at 45°C for four weeks; the mice were acclimatized to the laboratory environment for 1 week, then exposed to a temperature of 45°C and 50% humidity for 2h daily for 28 days. The signs of heat stress during the exposure were monitored. G1, mice treated with GA 10% extract for a week in drinking water; G2, mice treated with GA 10% extract for 28 days; G3, mice challenged with Ciptalin and treated with GA 10% extract for 28 days; G4, heat-stressed mice and treated with GA 10% extract for 28 days in drinking water.

Growth performance: Mice were weighed individually at the beginning and end of the study, and feed intake per cage was recorded daily. Body weight gain, feed conversion ratio and survival rate were calculated for each animal. Upon completion of the treatment periods, the mice were euthanized by cervical dislocation and the heart, brain, liver, kidneys, lungs, and spleen were dissected. After removing any excess fat, the relative weight of each organ was determined as a percentage of total body weight (Zhou *et al.*, 2024).

Blood biochemistry: At the end of the experiment (28 days), three mice were randomly selected from each group, and anesthesied using ether. The blood was collected from retro-orbital veins using capillary tubes. The blood samples were centrifuged at 2000-3000g for 10-15 min, and then the serum was collected. Serum activities of liver enzymes AST and ALT were measured using kits from Spectrum, Egypt. Similarly, serum urea and creatinine levels were determined using Spectrum kits, as described earlier (Burtis and Ashwood, 1999). Oxidative stress marker, MDA, and defense system markers CAT, POD, and SOD were measured using recommended kits (Al-Hazmi *et al.*, 2021). Serum levels of sex hormones (Testosterone, LH, and FSH) were measured following the methods described by Steyn *et al.* (2013). For testosterone, assay sensitivity was 0.1-0.2ng/mL, intra-assay CV was 3-8%, and inter-assay CV was 5-10%. For LH and FSH, assay sensitivity was 1-2mIU/mL, intra-assay CV was 3-7% and inter-assay CV was 5-9%.

Heat stress, proinflammatory, and precancerous markers estimation: Total RNA was extracted from kidney tissues collected from 3 mice of each group following the method described by Dong *et al.* (2020). The isolated RNA was subsequently used for quantitative real-time PCR (qRT-PCR) analysis. All qRT-PCR reactions were performed using SYBR Green chemistry on a real-time PCR system. Melting curve analysis was conducted to ensure PCR product specificity. Gene-specific primers for Hsp70, Hsp90, Bax, Caspase 3, and IL-6 (Table 1) were used for qRT-PCR. The 2-ΔΔCt method determined relative gene expression (Livak and Schmittgen, 2001).

Table 1: Forward and reverse primers sequence for Hsp70, Hsp90, Bax, Caspase 3, and IL-6 genes

Gene	Primer 5-3	MW (KDa)
Hsp70	F: ATCACCATCACCAACGAC R: ACTTGTCCAGCACCTTCTT	70
Hsp90	F: ATCACTGGTGAGAGCAAGAAGGC R: TTAGTCGACCTCCTCCATCTTGCT-3	90
Bax	F: ACACCTGAGCTGACCTTG, R: GCCCATGATGGTTCTGATC	21.1
Caspase 3	F: CTGAACTTCGGGGTGATCG, R: GCT TGG TGG TTT GCT ACG AC	17
IL-6	F: CCACTTCACAAGTCGGAGGCTTA R: CCAGTTTGGTAGCATCCATCATTTC	25

Molecular weight (MW).

Histopathological study: Kidney tissue samples (3 mice from each group) were fixed in 10% buffered formalin saline for histopathological study. Sections (0.5cm thick) were prepared using standard paraffin-embedding technique. Subsequently, paraffin specimens (5µm in thickness) were stained with hematoxillin and eosin (H&E) to assess kidney damage (Bancroft and Gamble, 2008). A semiquantitative scoring system was employed based on the percentage of tubules exhibiting epithelial necrosis, brush border loss, and cast formation, as follows: 0 (normal tubules), 1 (≤10% affected tubules), 2 (11-25% affected tubules), 3 (26-45%), 4 (46-75% affected tubules), and 5 (≥76% affected tubules). The average score was calculated for 10 randomly selected fields (Fu *et al.*, 2019).

Statistical analysis: The data are presented as mean±SE. For statistical analysis, the data were subjected to one-way ANOVA under completely randomized design (Snedecor and Cochran, 1989), using SPSS (version 22, USA). The comparison of multiple means was carried out by using LSD, and differences were considered statistically significant at $P<0.05$.

RESULTS

The active compound in GA extract: GC-MS analysis of a GA extract identified 13 diverse bioactive compounds. Table 2 summarizes the active compounds in GA extract with their molecular weight and their % area. The GC/MS profile of GA showed a variety of active compounds; the main compound was lycopene (4.50%), followed by 2,4,5- Trihydroxy pyrimidine (3.2%). The phenolic acid accounted for 1.9%, pigments 4.72%, and phenolic compounds 3%.

Table 2: GC-MS profile of active compounds in gum Arabic extract

Active compound	Chemical formula	% Area
Acetylenedicarboxylic acid	$C_4H_2O_4$	0.33±0.01
Butanedioic acid	$C_4H_6O_4$	0.25±0.02
Catechol	$C_6H_6O_2$	1.20±0.2
2,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	0.65±0.03
Benzoic acid-2-methyl pentyl ester	$C_{13}H_{18}O_2$	0.98±0.02
Oleic Acid	$C_{18}H_{34}O_2$	0.67±0.05
Lupulon	$C_{26}H_{38}O_4$	1.80±0.6
Astaxanthin	$C_{40}H_{52}O_4$	0.35±0.02
9-Octadecenal	$C_{18}H_{34}$	0.31±0.01
β-Carotene	$C_{40}H_{56}$	0.22±0.04
Lycopene	$C_{40}H_{56}$	4.50±0.9
2,4,5- Trihydroxypyrimidine	$C_5H_5NO_3$	3.20±0.2
Betamethasone	$C_{22}H_{29}FO_5$	1.77±0.8

Data are presented as mean±SE.

Biological activities of GA extract

Antioxidant activity: The phenolic compounds (Fig. 1A) and flavonoids (Fig. 1B) in GA extract increased steadily and significantly as the concentration of GA was increased from 2 to 10%. The 10% GA extract exhibited 59mg/kg phenolic compounds and 21mg/kg flavonoids. The scavenging activity against DPPH free radicals for 8 and 10% GA extract was comparable to that of AsA (Fig. 2). However, 2, 4 and 6% GA extract showed significantly lower antioxidant activity compared to AsA; the lowest activity was recorded for 2% GA extract ($P<0.05$). GA extract (10%) successfully scavenged 92% of DPPH free radicals with IC_{50} of 4% against DPPH free radicals.

Antimicrobial activity: This study tested the antibacterial and antifungal activity of GA against six phytopathogenic fungi and four bacteria (Table 3). The inhibition zones diameters (IZDs) increased as the concentration of GA extract was increased from 2 to 10%. GA 10% significantly reduced the growth of pathogenic bacteria with IZDs of 21.0±0.3 to 31.0±0.2mm for bacteria and 27.0±0.5 to 31.0±0.6mm for pathogenic fungi. *Klebsiella pneumonia* was resistant to GA 10%, while *Staphylococcus aureus* was the most susceptible. On the other hand, *A. niger*, and *P. aphanidermatum* had the lowest IZD of 27mm and *Alternaria alternata* had the highest IZD of 31mm (Table 3). The MIC values of GA against the test pathogens ranged between 0.5-and 2% (4.9-19.9µg/mL) (Fig. 3).

The SEM images of control bacteria and fungi showing their normal smooth texture are shown in Fig. 4A and Fig. 4C, respectively. Treatment with GA extract showed antimicrobial activity against bacteria and fungi. It disrupted the cell integrity of both types of microorganisms. This disruption was evident in the bacterial cells by the rough and damaged surfaces (Fig. 4B), with some cells appeared to have burst. In the fungal cells, the hyphae showed signs of collapse and shriveling (Fig. 4D).

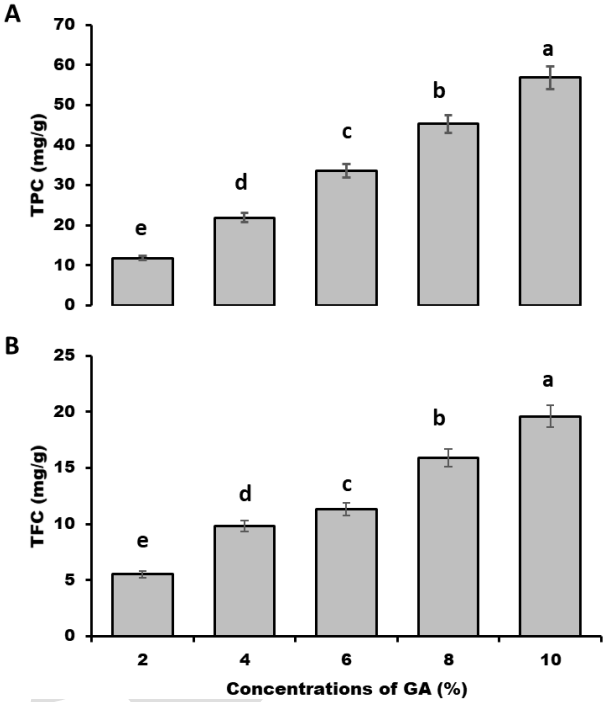


Fig. 1: (A) Total phenolic content (TPC), and (B) total flavonoids content (TFC) in different concentrations of gum Arabic extract. Different letters above columns indicate significant differences ($P<0.05$).

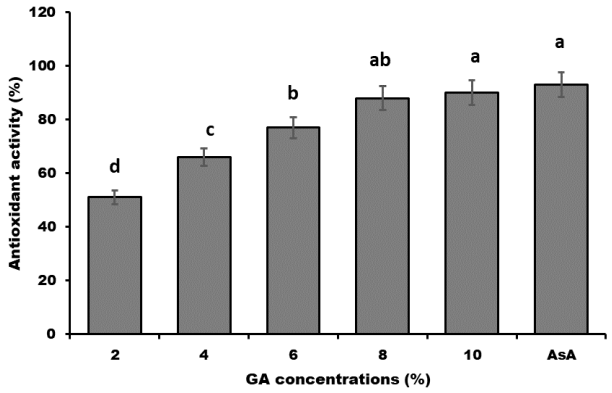


Fig. 2: Antioxidant activity of different concentrations of gum Arabic extract against DPPH radicals. Different letters above columns indicate significant differences ($P<0.05$).

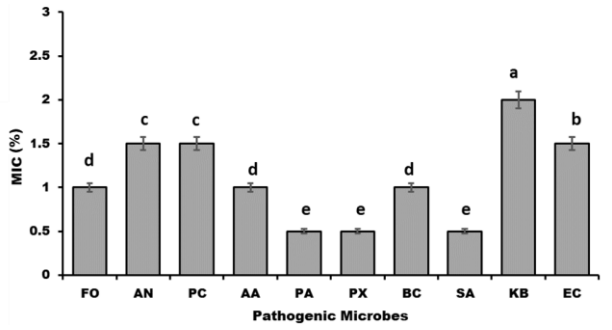


Fig. 3: MIC values of gum Arabic extract against pathogenic bacteria and fungi. Different letters above columns indicate significant differences ($P<0.05$). *Fusarium oxysporum* (FO), *Aspergillus niger* (AN), *P. chrysogenum* (PC), *Alternaria alternata* (AA), *P. aphanidermatum* (PA), *Podosphaera xanthii* (PX), *Bacillus cereus* (BC), *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Escherichia coli* (EC).

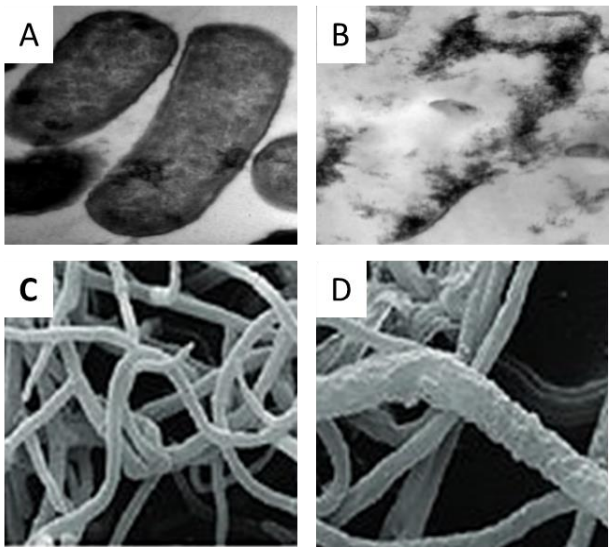


Fig. 4: SEM images for the antimicrobial effect of GA extract on the pathogenic bacteria and fungi. (A) control bacterial cell; (B) GA-treated bacterial cell showing the cell wall damage; (C) control fungal cell, and; (D) GA-treated fungal cells showing torn hypha and cell wall.

Table 3: Antimicrobial activity in terms of IZD (mm) of gum Arabic extract (2-10%) against pathogenic microorganisms (data are mean±SE)

Microorganisms	GA concentration (%)				
	2	4	6	8	10
Pathogenic bacteria					
Bacillus cereus	12±0.2bE	15±0.5bD	18±0.8bC	22±0.5bB	27±0.3bA
Staphylococcus aureus	15±0.3aE	19±0.6aD	23±0.6aC	26±0.6aB	31±0.2aA
Escherichia coli	11±0.6bcE	14±0.8bcD	17±0.5bcC	21±0.2bcB	25±0.8cA
Klebsiella pneumonia	10±0.3cE	13±0.5cD	15±0.4cC	18±0.1cB	21±0.3dA
Pathogenic fungi					
Fusarium oxysporum	15±0.2bE	20±0.3bD	23±0.2bC	26±0.2bB	30±0.3bA
Aspergillus niger	13±0.3cD	18±0.2cC	21±0.5cB	25±0.3bcAB	27±0.5cA
P. chrysogenum	15±0.6bE	18±0.5cD	23±0.3bC	25±0.5bcB	29±0.5bcA
Alternaria alternata	18±0.5aD	22±0.9aC	25±0.6aB	29±0.6aAB	31±0.6aA
P. aphanidermatum	14±0.0bcE	17±0.9dD	20±0.6dC	23±0.5cB	27±0.2cA
Podosphaera xanthii	14±0.0bcE	18±0.2cD	21±0.6cC	25±0.5bcB	28±0.6bcA

Values with different lowecase letters in the same column for each microorganism type (bacteria or fungi), and uppercase letters in the same row indicate significant differences (P<0.05).

Anticancer activity: Figure 5 shows the anticancer activity of GA at different concentrations against breast cancer cell lines. Microscopic image of MCF-7 cancer cell lines is shown in Fig. 5A. The growth inhibition of MCF-7 by 2% (Fig. 5B) and 10% (Fig. 5C) GA extract compared to doxorubicin (DOX, 300µg/mL a spositive control (Fig. 5D) has been presented. The viability percentage of MCF-7 cell lines decreased significantly (P<0.05) with the increase in concentration of GA extract. The lowest cell viability (23%) was recorded for DOX 300µg/mL, followed by 25% cell viability for 10% GA extract, while the control group showed the highest cell viability of 100% (Fig. 5E).

Antidiabetic activity: The inhibition of activity of two enzymes, α-amylase and α-glucosidase, significantly (p<0.05) increased as the concentration of GA extract was increased. The activity of α-amylase was inhibited by 78% (Fig. 6A), while that of α-glucosidase by 85% (Fig. 6B) compared to control group. The inhibition of α-amylase and α-glucosidase is relevant for managing diabetes, as these enzymes are involved in carbohydrate digestion.

Growth parameters: Table 4 shows that cisplatin and heat-challenged mice showed the lowest body weight gain (BWG) of 44.7±0.9 and 47.1±0.5g, respectively, with relative decreases of 22.3 and 25% compared to control group (P<0.05). The G1 and G2 mice exhibited the highest BWG, while the combined treatment wit GA mitigated the impact of cisplatin and heat stress, enhancing the BWG by 24 and 28% compared to PC1 and PC2, respectively (P<0.05). There was a significant decrease in FCR values in G1, G2, G3 and G4 groups, compared to PC1 and PC2, the latter two grousp showed the highest FCR values (P<0.05). Survival rates were the same in all groups, while relative organ weight was the highest in PC1 and PC2, and decreased in G1, G2 and G4 (P<0.05).

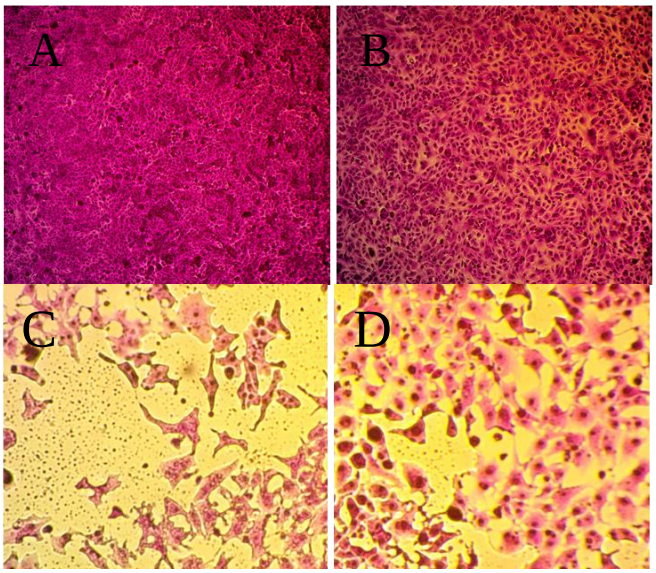
Biochemical incidences of blood : As shown in Table 5, cisplatin and heat challenged groups showed significant elevation in liver enzymes (ALT & AST) activities compared to negative controls. However, the inclusion of GA in the diet of these mice effectively regulated these enzyme activities, causing decrease of 47 and 55.2% for ALT and 39 and 59% for AST in G3 and G4, respectively. Uera levels were elevated in PC1 and PC2 groups but significantly decreased in G1, G2, G3 and G4. However, creatinine levels were unaffected with different treatments (Table 5).

The heat and cisplatin-challenged group exhibited elevated TC, TG and LDL levels, with reduced HDL levels compared to negative controls (P<0.05). Dietary GA supplementation significantly improved the lipid profile. In groups G4, TC, TG, and LDL levels were reduced by 43, 50, and56 %, respectively; however, HDL increased by 78%. The serum glucose and insulin hormone were upregulated in PC1 and PC2 groups, while adding GA 10% downregulated these levels.

Finally, the addition of GA to the drinking water of mice significantly enhanced oxidative stability compared to the heat and cisplatin-challenged groups (P<0.05). This was evidenced by decreased MDA levels and a concomitant increase in defense system activity (antioxidant enzymes). The levels of reproductive hormones were decreased in PC1 and PC2 groups compared to negative control (P<0.05). However, these levels weresignificantly improved following GA extract treatment (Table 5).

Proinflammatory heat stress and proapoptotic markers: As shown in Table 6, PC1 and PC2 groups showed significant increase in IL-1β and TNF-α compared to negative control group, while G1 and G2 showed levels close to NC (though significantly higher in G2). However, the combined GA treatments in G3 (Cisplatin+GA 10%) and G4 (Heat Stress+GA 10%) groups showed downregulation in both IL-1β and TNF-α compared to PC1 and PC2. This suggests that GA partially mitigates the inflammatory response caused by cisplatin and heat stress, although levels were still higher than NC.

Regarding the heat stress proteins, PC1 (Cisplatin) and PC2 (Heat Stress) showed downregulation in HSP70 and HSP90 genes compared to NC. G1 (GA 2%) and G2 (GA 10%) groups showed significant increase in HSP70



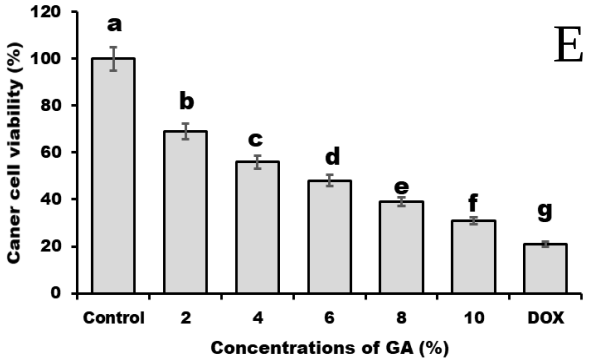


Fig. 5: (A) Microscopic image of MCF-7 cancer cell lines; (B) photograph of the cytotoxicity effect of GA 2% on the MCF-7 viability; (C) the cytotoxicity effect of GA 10% on the MCF-7 viability; (D) the cytotoxicity of doxorubicin (DOX, 300µg/mL) as positive control on MCF-7 viability; (E) Histogram of the effect of GA concentrations on the viability of MCF-7 compared to DOX. Different letters above columns indicate significant differences (P<0.05).

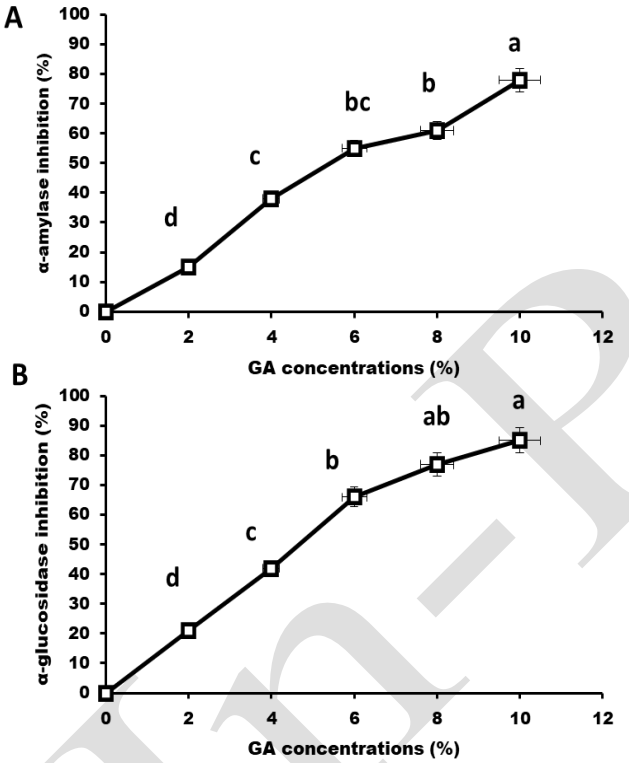


Fig. 6: The percentage of inhibition activity of different concentrations of GA extract against α-amylase enzyme (A), and against α-glucosidase enzyme (B). Lowercase letters (a-d) above each point indicate significant differences (P<0.05).

and HSP90, especially at 10% GA. On the other hand, the combined treatment groups, G3 (Cisplatin+GA) and G4 (Heat stress+GA), showed a partial recovery in HSP70 and HSP90 levels compared to PC1 and PC2 (Table 6).

The precancerous markers (BAX and Casp-3) affected by different treatments are shown in Table 6, where PC1 (Cisplatin) and PC2 (Heat Stress) showed a significant increase in BAX and Casp-3 compared to NC (P<0.01), indicating an increase in apoptosis due to cisplatin and heat stress. While G1 (GA 2%) and G2 (GA 10%) showed levels comparable to NC group, suggesting that GA alone did not induce apoptosis. However, G3 (Cisplatin+GA) and G4 (Heat stress+GA) groups showed a significant reduction in precancerous markers compared to PC1 and PC2. This indicates that GA partially mitigates the apoptotic effects of cisplatin and heat stress.

Renal histopathology: Histopathological examination of kidneys in negative control group (Fig. 7A) revealed intact glomeruli with well-defined Bowman's capsules and mesangial cells. The tubules had a clear lumen and intact epithelial lining. The interstitial tissue was minimal without any signs of inflammation or congestion. The cisplatin-challenged group (PC1) showed tubular necrosis, indicated by the loss of the tubular epithelial lining, sloughing of cells into the tubular lumen and cellular debris. Some glomeruli appeared shrunken. Casts were visible within the renal tubules. Overall, the architecture was disrupted with signs of severe injury (Fig. 7B). The heat-stressed group (PC-2) exhibited vacuolization in the tubular epithelial cells, suggesting cellular stress or damage (Fig. 7C). Some tubules were dilated, with mild interstitial inflammation. The glomeruli, however, appeared normal compared to the cisplatin group.

Kidneys of GA 2% (G1) group appeared normal, similar to the control (Fig. 7D. This suggests that GA at 2% concentration had no adverse effect on the kidney. The same was true for the mice of group G2 treated with GA 10% (Fig. 7E).

In the cisplatin-challenged group treated with 10% GA (G3), kidneys showed a noticeable improvement compared to the PC1 (cisplatin-treated) group. The tubular damage was less severe, with less cellular debris and fewer casts in the tubules. The glomeruli appeared relatively better preserved (Fig. 7F). This suggests that GA might offer some protection against cisplatin-induced nephrotoxicity. The kidneys in heat-stressed group treated with 10% GA (G4) also showed some improvement compared to the heat-stress-only group (PC2). The vacuolization in the tubular epithelial cells appeared reduced, and the overall architecture was more intact (Fig. 7G). This suggests that GA had mitigated the damaging effects of heat stress on the kidneys.

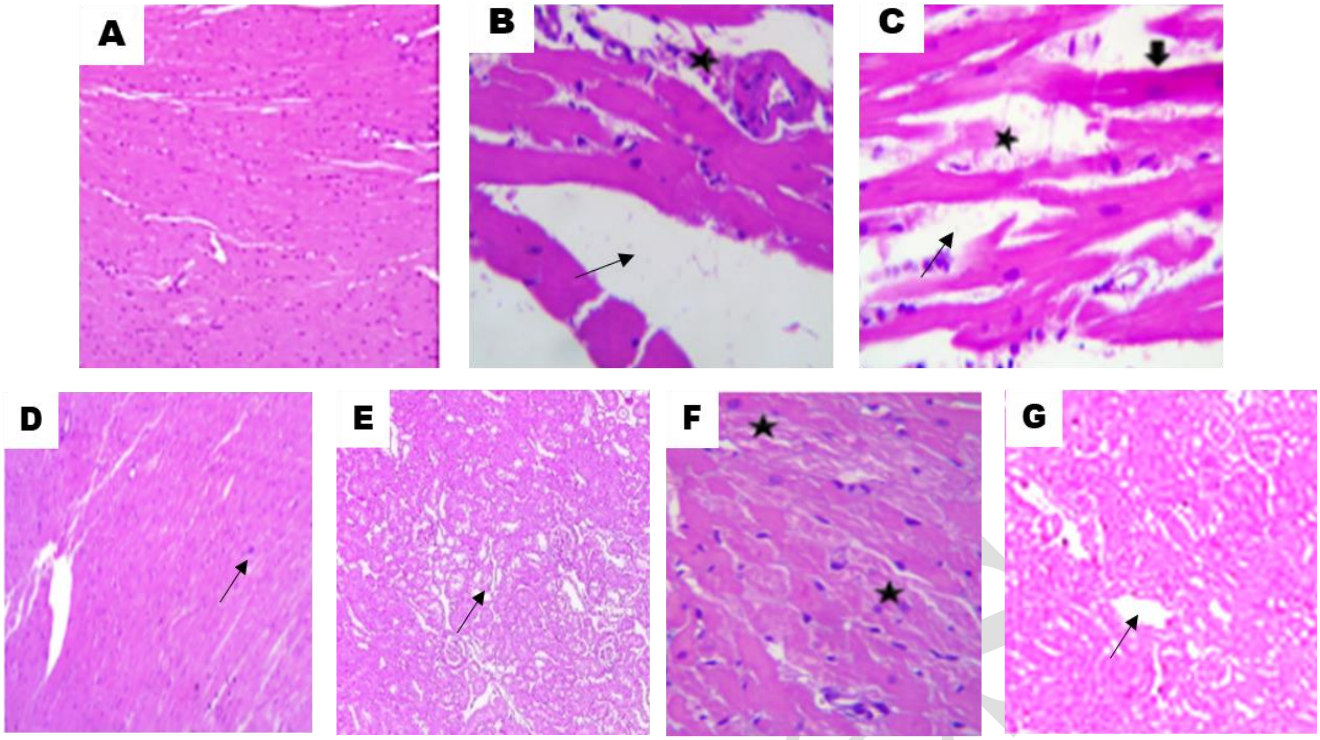


Table 4: Effect of dietary treatments of GA extract on growth performance parameters of mice under ciptalin and heat-stress (data are mean±SE).

Parameters	NC	PC1	PC2	G1	G2	G3	G4
LBW (g)	30.1±0.1	30.5±0.0	30.6±0.1	31.3±0.0	31.6±0.2	31±0.1	30±0.2
FBW (g)	88.7±1.1c	75.2±0.9d	77.7±1.1d	95.3±1.3b	106.8±1.9a	88.3±0.9c	89.9±0.8c
BWG (g)	58.7±0.8c	44.7±0.9d	47.1±0.5d	64±0.8b	75.2±0.9a	57.3±0.9c	59.9±0.8c
FCR	1.51±0.2b	1.68±0.1a	1.62±0.3a	1.48±0.2c	1.42±0.2c	1.54±0.1b	1.50±0.3b
SR (%)	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
ROW (%)	2.45±0.2c	3.9±0.5a	3.5±0.4ab	2.51±0.2c	2.53±0.6c	3.0±0.2b	2.8±0.5bc

Values with different letters within a row differ significantly (P<0.05). Live body weight (LBW); Final body weight (FBW); weight gain (BWG), Feed intake (FI), Feed conversion ratio (FCR); survival rate (GR), and relative organ weight (ROW). NC, negative control delivered basal diet without any stressors; PC1, positive control, delivered basal diet and challenged with Ciptalin; PC2, positive control, delivered basal diet under heat stress 45°C; G1, mice treated with GA 10% for a week; G2, mice treated with GA 10% for 28 days; G3, mice challenged with Ciptalin and treated with GA 10% for 28 days; G4, heat-stressed mice and treated with GA 10% for 28 days.

Table 5: Effect of dietary treatments with GA extract on blood biochemistry, reproductive hormones, and antioxidant status of mice under ciptalin and heat-stress (data are mean±SE)

Serum parameters	Treatments groups						
	NC	PC1	PC2	G1	G2	G3	G4
Liver and Kidney functions							
AST (U/L)	120±1.3d	259±2.1a	250±2.2a	110±1.2e	106±1.3e	158±1.4b	148±1.1c
ALT (U/L)	158±1.6c	315±3.3a	230±2.6a	145±1.2d	141±1.3d	167±1.5b	155±1.1c
Creat. (mg/dL)	0.32±0.3	0.35±0.0	0.35±0.2	0.31±0.1	0.33±0.6	0.36±0.2	0.33±0.3
Urea (mg/dL)	5.3±0.1c	7.4±0.2a	6.9±0.3b	3.9±0.6e	3.6±0.2e	4.8±0.6d	4.3±0.5d
Lipid profile							
TC (mg/dL)	74±1.1cd	135±2.0a	120±1.3b	65±1.6d	61±0.9d	79±0.5c	77±0.9c
TG (mg/dL)	45±0.2d	90±0.6a	81±0.6b	37±0.2e	33±0.6e	51±0.9c	45±0.6d
LDL (mg/dL)	21±0.3c	50±0.6a	45±0.3b	18±0.9d	15±0.6d	23±0.5c	22±0.8c
HDL (mg/dL)	36±0.9c	18±0.2e	21±0.6d	55±0.6a	49±0.6b	31±0.8cd	32±0.9cd
Blood glucose							
Glucose (mmol/L)	9.7±0.2b	15.3±0.6a	12.6±0.6ab	5.5±0.8c	5.1±0.7c	9.9±0.6b	9.7±0.5b
Insulin (mIU/L)	21.5±0.2c	55.3±0.9a	48.6±0.8ab	20.6±0.6c	21.1±0.5c	28.3±0.8b	27.1±0.7b
Antioxidant status							
MDA (nmol/mL)	2.3±0.2bc	7.8±0.3a	7.1±0.2a	0.31±0.03c	0.33±0.01c	2.8±0.2b	2.5±0.2b
SOD (U/mL)	3.6±0.3b	2.1±0.5c	2.3±0.6c	6.1±0.2a	6.5±0.2a	3.0±0.6bc	3.2±0.3b
CAT (U/mL)	5.3±0.5bc	3.2±0.3c	3.5±0.1c	7.5±0.6a	7.7±0.6a	5.9±0.6b	5.5±0.5b
GPx (U /mL)	27±0.9b	12±0.6d	15±0.6d	33±0.9a	36±0.7a	21±0.9c	23±0.6c
Sex hormones							
Testosterone (ng/mL)	0.9±0.01b	0.02±0.001c	0.09±0.002c	1.3±0.2a	1.5±0.2a	1.1±0.01b	0.9±0.02b
LH (ng/mL)	1±0.1b	0.1±0.02e	0.5±0.03d	2.5±0.6a	2.8±0.3a	1.0±0.02b	0.88±0.01c
FSH (ng/mL)	1.5±0.2c	0.5±0.02d	0.82±0.01d	20±0.2a	21±0.9a	9±0.1b	7.8±0.6b

Values with different letters within a row for each parameter differ significantly (P<0.05). AST, aspartate transaminase; ALT, alanine transaminase; creat., creatinine; Total cholesterol (TC); triglycerides (TG); low-density lipoprotein (LDL); high-density lipoprotein (HDL); malondialdehyde (MDA); superoxide dismutase (SOD); catalase (CAT), peroxidase (GPx).

Table 6: The effect of dietary treatments GA extract on the fold change in proinflammatory cytokines, precancerous markers, and heat stress protein in mice under cisptalin and heat-stress (data are mean±SE)

Genes	Proinflammatory markers (Fold change)		Heat stress protein (Fold change)		Precancerous markers (Fold change)	
	IL-1β	TNF-α	HSP70	HSP90	BAX	Casp-3
NC	1.1±0.2d	1±0.1d	20±0.8c	15±0.6b	1±0.1e	1.04±0.5d
PC1	10.9±0.6a	9.8±0.3a	11±0.6d	8±0.2d	13±0.3a	7.8±0.5a
PC2	9.6±0.9ab	9.3±0.6ab	13±0.5d	10±0.3c	11±0.6a	7.1±0.3a
G1	1.2±0.2d	1.1±0.2cd	45±0.9ab	25±0.6ab	1.4±0.6d	1.1±0.5c
G2	2.3±0.3c	1.5±0.6c	49±0.6a	30±0.5a	2.3±0.5c	1.7±0.8c
G3	4.1±0.2b	3.7±0.2b	17±0.5b	11±0.4c	5.1±0.5b	3.0±0.2b
G4	3.8±0.3bc	3.5±0.6b	18±0.6b	13±0.6bc	4.9±0.8b	2.6±0.6b

Values with different letters within a column differ significantly (P<0.05).

DISCUSSION

Oxidative stress, caused by an imbalance between free radicals and antioxidants, can damage cells and contribute to various diseases (El-Kassas *et al.*, 2022; Alsulami and El-Saadony, 2024). Phytogenic compounds naturally combat oxidative stress (Reda *et al.*, 2024a,b). These compounds, including phenolic compounds, flavonoids, polysaccharides, and essential oils (El-Tarabily *et al.*, 2021), possess potent antioxidant properties that neutralize free radicals and protect cells from damage (Abd El-Hack *et al.*, 2022a; Arif *et al.*, 2022).

Phytogenic compounds are known to naturally combat oxidative stress (Reda *et al*., 2024a,b). These compounds such as phenolics, flavonoids, polysaccharides, and essential oils (El-Tarabily *et al*., 2021) exhibit strong antioxidant properties that help neutralize free radicals and protect cells from damage (Abd El-Hack *et al*., 2022a; Arif *et al*., 2022).

The use of phytogenic compounds as antioxidant agents is increasingly recognized globally due to their potential benefits and safety profile compared to synthetic antioxidants (Abd El-Hack *et al*., 2022b; Soliman *et al*., 2024). These compounds have demonstrated promising effects in preventing chronic diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. Moreover, they can be integrated into functional foods and dietary supplements to enhance overall health and well-being (Saad *et al*., 2021a,b; El-Sadoony *et al*., 2024a,b).

This study focused on exploring the protective effects of GA against cisplatin-induced nephrotoxicity and understanding the underlying mechanism. The results revealed that oral administration of 10% GA in drinking water significantly alleviated heat and cisplatin-induced kidney injury. This protective effect was reflected in improved serum creatinine and urea levels, reduced oxidative stress marker (MDA), and increased antioxidant enzyme levels (SOD, CAT, and GPx). Histopathological findings further corroborated these results.

In this study, rats injected with cisplatin exhibited a notable decrease in body weight, likely due to disruptions in energy production and lipid metabolism (Conte *et al*., 2020). These observations partially align with the findings of Bushra and Bastwrous (2022), who reported body weight reduction in male Albino rats following cisplatin injection (7.5 mg/kg, i.p.) treatment with GA mitigated their weight loss. However, Di Francesco *et al*. (2024) found that GA treatment over 14 consecutive days did not influence body weight. In contrast, Dakhil and Alhsan (2020) observed a reduction in weight loss when GA was admisinstered in drinking water.

In this study, cisplatin administration led to significant increase in serum creatinine and urea levels, indicating acute renal failure (Zaaba *et al*., 2022). This nephrotoxicity is linked to several mechanisms, including cisplatin uptake by renal epithelial cells, damage to nuclear and mitochondrial DNA, activation of cell death pathways, and induction of a strong inflammatory response and oxidative stress (Xi *et al*., 2023).

These events ultimately impair kidney function, reducing the ability to eliminate nitrogenous waste products from the blood (McSweeney *et al*., 2021; Zhang *et al*., 2023). These results align with the findings of Kokhdan *et al*. (2021), who reported that a single intraperitoneal injection of cisplatin at a high dose (7.0 mg/kg) caused nephrotoxicity lasting three days, marked by a decreased glomerular filtration rate, elevated serum creatinine and blood urea nitrogen (BUN) levels, and impaired urine concentration ability.

The administration of 10% GA in drinking water alongside cisplatin significantly reduced the cisplatin-induced rise in serum creatinine and urea levels. This renoprotective effect of GA is likely due to its ability to lower urea levels by increasing fecal excretion of urea nitrogen while reducing urinary urea nitrogen excretion (Ali *et al*., 2020). These findings are consistent with the previous studies (Tain *et al*., 2023; Barkeer *et al*., 2024), which showed that long-term GA administration in drinking water restored kidney marker levels altered by adenine injection.

In this study, a significant decrease in antioxidant enzyme levels (CAT, SOD, and GPx) was observed in mice treated with a high dose of cisplatin (4.5 mg/kg) for two consecutive days. This reduction can be attributed to several mechanisms, including the generation of reactive oxygen species by cisplatin (McSweeney *et al*., 2021). Cisplatin is biotransformed by the cytochrome-P450 system, producing highly reactive thiol compounds. This process depletes glutathione, contributing to cellular damage (Potęga, 2022)

A significant elevation in blood MDA levels was observed following cisplatin treatment, indicating increased lipid peroxidation. This disruption of the antioxidant defense system and increased oxidative stress are the major contributors to cisplatin-induced kidney injury. Intracellularly, cisplatin is rapidly converted into a highly reactive species that readily reacts with glutathione. This glutathione depletion further exacerbates oxidative stress within the cells. Moreover, mitochondrial dysfunction, a major source and target of ROS, plays a crucial role in the generation and propagation of oxidative stress in cisplatin nephrotoxicity (Fang *et al.*, 2021; Su *et al.*, 2023).

Administration of 10% GA extract together with cisplatin in drinking water significantly restored MDA and antioxidant enzymes (CAT, SOD, and GPx) levels to their normal values. This supports findings of the previous studies (Alobaidi, 2024; Habashy and Abu-Serie, 2024), demonstrating GA antioxidant activity, including its potent superoxide scavenging action, which protects against nephrotoxicity. GA treatment significantly

elevated the levels of HSP70 and HSP90 proteins within muscle tissue. These findings corroborate with the previous research demonstrating that increased heat shock protein levels are associated with reduced oxidative stress, thereby protecting tissues from cellular damage (Chauhan *et al.*, 2021).

Gum Arabic is rich in phytochemicals, including flavonoids, alkaloids, tannins, terpenoids, and saponins. These compounds, particularly saponins and flavonoids, have been shown to enhance fertility. Stress leads to increased levels of reactive oxygen species, which can cause erectile dysfunction by damaging Leydig cells in the testes. This damage can reduce testosterone production and lead to decreased libido. The antioxidants of phytochemicals in GA can help protect erectile tissues from oxidative damage. Saponins have anti-stress properties, helping the body cope with stress (Shehab-El-Deen *et al.*, 2022; Zhang *et al.*, 2022).

Furthermore, both saponins and flavonoids have been implicated in promoting penile erection. Testosterone plays a crucial role in erectile function. However, oxidative stress caused by ROS can impair testosterone production (Zhang *et al.*, 2022).

Histopathological examination revealed that cisplatin administration resulted in severe tubular damage, characterized by swelling, necrosis, desquamation of the tubular epithelium, loss of brush borders, intratubular cast formation, and cystic dilatation, consistent with previous findings (Çetinavci *et al.*, 2022).

Cisplatin, a small, neutral molecule, is freely filtered by the glomerulus and readily reabsorbed by the renal tubules. This high intratubular concentration, particularly within the proximal tubules of the inner cortex and outer medulla (S3 segment), contributes to the tubular damage observed in this study. However, co-administration of 10% GA extract significantly ameliorated these histopathological alterations. This demonstrated that the GA-treated group exhibited milder cystic dilatation and degeneration than the cisplatin-treated group. Notably, the brush border integrity was predominantly preserved in the GA-treated mice. These findings align with those of Bashraf *et al.* (2021) and Kandeal *et al.* (2021), who demonstrated that pre-treatment or concomitant GA administration with cisplatin improved histopathological outcomes, including preserved glomerular and convoluted tubule architecture. Thus, there is a clear indication to use natural compounds with distinct biological activities i.e., antioxidant (Abdelbaky *et al.*, 2023), anticancer (Mueed *et al.*, 2023), antiviral (Hegazy *et al.*, 2024), antidiabetic (Mueed *et al.*, 2024) and antimicrobial (Abd El-Hack *et al.*, 2022c) and their potential in mitigating the oxidative stress in livings caused by different conditions such as climate change and pollution by chemicals (Alharbi *et al.*, 2024).

Conclusions: This study reveals the efficacy of oral administration of GA extract in suppressing nephrotoxicity induced by cisplatin and heat stress. The observed antioxidant and anti-inflammation potential of GA suggests its potential as a promising therapeutic agent for protecting kidneys from the harmful effects of toxic agents, including those used in chemotherapy. By mitigating kidney damage, GA may delay the onset of kidney failure, minimize damage to kidney cells, and reduce associated complications. These findings highlight the potential clinical significance of GA as a supportive therapy in conjunction with cisplatin-based chemotherapy.

Authors' contributions: Conceptualization, NSA, KBS, MHAZ, and AAA, formal analysis, GMSAA, WAA, and MAM, investigation, MAA, AM, and MS, data curation, NSA, KBS, MHAZ, and AAA, writing original draft preparation, GMSAA, WAA, and MAM, writing final manuscript and editing, MAA, AM, and MS, visualization and methodology, NSA, KBS, MHAZ, AAA, GMSAA, WAA, MAM, MAA, AM, and MS. All authors have read and agreed to the final version of the manuscript.

Competing interests: The authors declare that they have no competing interests.

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