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Research Article

Evaluation of Gum Arabic Extract for Enhancing Growth Performance, Blood Parameters, Oxidative Status, Gut Microbiota, and Organ Histology in Heat-Stressed Rabbits

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Abstract

The study investigated the bioactive properties and effects of gum arabic (GA) on various biological parameters, including antioxidant, antimicrobial, anticancer, and growth-promoting activities, as well as its impact on blood biochemistry, oxidative status, immunity, and gut microbiota in heat-stressed rabbits. The main compounds in the GC/MS profile of GA were saccharides, i.e., arabinose, galactose, and rhamnose accounted for 41%, followed by phenolic acids with 36.1%. GA extract (10 %) contains 66 mg/g phenolic compounds and 24 mg/g flavonoids, demonstrating a significant % antioxidant activity of 91% and potent antimicrobial activity against pathogenic microorganisms; it also showed promising anticancer activity, reducing MCF-7 cancer cell viability by 88%. In growth performance studies, GA supplementation significantly enhanced the body weight gain, growth rate, feed conversion ratio, and performance index by 19, 18, 10, and 30% compared to the control. GA improved red blood cell production, liver function, and lipid metabolism. GA treatments significantly increased red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration by 5-15% compared to the control. Albumin, total protein, and globulin levels increased by 10-20%, indicating improved protein synthesis and liver function. Liver and kidney parameters, such as ALT, AST, creatinine, urea, triglycerides, glucose, total cholesterol, and LDL, decreased by 20-40%, while HDL cholesterol increased by 15-25%, suggesting enhanced lipid metabolism and reduced oxidative stress. GA supplementation also enhanced antioxidant defense systems, increasing total antioxidant capacity, superoxide dismutase and glutathione peroxidase (GSH-Px) by 20-30% while reducing malondialdehyde (MDA) levels by 40-50%. Immunoglobulin A, G, and M levels increased by 25-35%, indicating improved immunity. GA 10% concentration significantly reduced proinflammatory cytokines (IL-1β by 78% and TNF-α by 82%) and precancerous markers (BAX and Casp-3 by 75-80%) in heat-stressed rabbits while increasing heat stress proteins (HSP70 by 291% and HSP90 by 130-210%). Histological examinations revealed that GA preserved liver and kidney integrity under heat stress, with 6% and 10% concentrations showing near-normal tissue architecture compared to heat-stressed rabbits displayed cuboidal epithelial cell necrosis and exfoliation of the brush border. Additionally, GA positively modulated gut microbiota, reducing harmful bacteria like *E. coli* and Salmonella by 25-40% while increasing beneficial lactic acid bacteria by 45%. It concluded that GA supplementation enhanced the growth, blood biochemistry, and immunity of heat-stressed rabbits.

Keywords: Antioxidants, Blood, Cancer, Gum arabic, Heat stress, Rabbit

Introduction

Heat stress is a significant challenge in rabbit farming, particularly in regions with high ambient temperatures [1]. Rabbits are susceptible to heat due to their limited ability to dissipate body heat, as they lack sweat glands and rely primarily on panting and ear vasodilation for thermoregulation [2]. When environmental temperatures exceed their thermoneutral zone (15-25°C), rabbits experience heat stress, leading to a cascade of physiological,

metabolic, and immunological disruptions. This problem is exacerbated by high humidity, poor ventilation, and overcrowding in farming systems. Heat stress not only compromises the health and welfare of rabbits but also reduces productivity, making it a critical issue for the rabbit industry [3].

Heat stress, including physiological, metabolic, and immunological functions, negatively impacts rabbits, disrupting thermoregulation and leading to elevated body temperature, increased respiratory rate, and dehydration. These changes strain the cardiovascular system, reducing blood flow to vital organs and impairing nutrient and oxygen delivery [4]. Prolonged heat stress can cause heatstroke, organ failure, and even mortality. Heat-stressed rabbits exhibit reduced feed intake and altered metabolism [5]. To cope with heat, they shift energy utilization from growth to maintenance, leading to weight loss, poor growth performance, and reduced feed efficiency.

Additionally, heat stress disrupts lipid and glucose metabolism, increasing the risk of metabolic disorders. Also, it suppresses the immune system, making rabbits more susceptible to infections and diseases. It reduces the production of immunoglobulins and antioxidants, impairing the body's ability to combat oxidative stress and inflammation. This weakened immunity increases mortality rates and reduces overall productivity [6,7]. The reproductive performance reduced in both male and female rabbits. In males, it reduces sperm quality and quantity, while in females, it disrupts estrus cycles, decreases conception rates, and increases embryonic mortality. These effects lead to lower breeding efficiency and reduced litter sizes. The heat-stressed rabbits exhibit behavioral changes such as lethargy, reduced activity, and altered feeding patterns. These changes further exacerbate the negative impacts on growth and reproduction [8].

The conventional solutions focus on environmental modifications and management practices, such as providing well-ventilated housing with proper insulation to help maintain a cooler environment. Fans, misting systems, and evaporative coolers are commonly used to reduce ambient temperature and humidity. Installing shade structures and cooling pads in rabbit housing can help lower temperatures [9,10]. Access to cool, clean water is also essential to prevent dehydration. Feeding rabbits during cooler days and providing high-energy, easily digestible diets can help mitigate the effects of reduced feed intake during heat stress. Also, breeding heat-tolerant rabbit breeds is a long-term strategy to improve resilience to high temperatures [9,10]. Conventional methods are somewhat effective but often involve high costs, energy consumption, and infrastructure requirements, making them less accessible to small-scale farmers. Additionally, they may not fully address the physiological and metabolic challenges heat stress poses.

In recent years, alternative solutions, particularly nutritional interventions, and natural supplements, have gained attention due to their potential to mitigate heat stress in rabbits. These cost-effective and sustainable approaches address the root causes of heat stress at the cellular and metabolic levels [9,10].

Heat stress generates excessive reactive oxygen species (ROS), leading to oxidative damage. Supplementing diets with antioxidants such as vitamin C, E, and selenium can neutralize ROS, reduce oxidative stress, and improve overall health [11,12]. For example, studies have shown that vitamin E supplementation enhances immune function and reduces mortality in heat-stressed rabbits. Also, probiotics (beneficial bacteria) and prebiotics (dietary fibers that promote probiotic growth) improve gut health and nutrient absorption. They enhance the gut microbiota's resilience to heat stress, reduce inflammation, and boost immunity. Probiotic supplementation has been shown to improve growth performance and reduce the incidence of diarrhea in heat-stressed rabbits [13].

On the other hand, herbal extracts such as ginger, turmeric, and moringa possess anti-inflammatory, antioxidant, and immunomodulatory properties. These natural compounds can mitigate the adverse effects of heat stress by enhancing antioxidant capacity, reducing inflammation, and improving metabolic efficiency [14]. Supplementing diets with omega-3 fatty acids, found in flaxseed and fish oil, can reduce inflammation and strengthen thermoregulation in heat-stressed rabbits. These fatty acids enhance cell membrane integrity and support immune function. Providing electrolyte solutions helps maintain hydration and electrolyte balance, which is critical during heat stress. Electrolytes such as sodium, potassium, and magnesium support cardiovascular function and nutrient transport [15].

Arabic gum (GA) is a natural, complex substance derived from the sap of Acacia trees, primarily Acacia Senegal and Acacia seyal. This branched-chain hydrocolloid consists mainly of arabinose and galactose sugars, proteins, and minerals like calcium, magnesium, and potassium. With a neutral or slightly acidic nature, GA was employed as an adhesive for mummification and in mineral paints for hieroglyphs [16]. Today, GA finds widespread application in various industries, including food, pharmaceuticals, and many others. The main medicinal uses of GA include respiratory disorders, gastrointestinal issues, liver and kidney health, and skin and inflammatory disorders. Additionally, GA extract has been used to treat chronic hepatitis and has therapeutic benefits against various viruses [16-18].

Studies have found that GA extract exhibits potent antibacterial activity against standard *S. aureus* and MRSA

strains by reducing lipase activity and inhibiting virulence gene expression [19]. Arabic gum has the potential to accelerate burn wound healing through the following properties: anti-inflammatory effects, antimicrobial properties, antioxidant activity, wound contraction and shrinkage, reduced pain and discomfort, faster epithelialization, and enhanced angiogenesis [20]. Abd El-Azeem et al.[21] demonstrated that supplementing diets with turmeric, MOS, and Biostrong significantly improved doe weight, milk yield, birth size, antioxidant capacity (TAC, GPx, SOD), and protein levels while reducing oxidative stress (MDA), highlighting their effectiveness as natural antioxidants in mitigating heat stress during pregnancy and lactation in rabbits. To the best of our knowledge, this is the first study to investigate the effects of GA extract on heat stress in rabbits; therefore, in this study, the active compounds in GA extract were detected using GC-MS, then examined its antioxidant, anticancer, and antimicrobial activity, elucidating the effect of GA extract on the heat stressor, monitoring the biochemical, molecular and histological changes.

Materials and Methods

Ethical Approval

The Unit of Biomedical Ethics has approved the animal study, Research Ethics Committee (REC HA-02-J-008, King Abdul Aziz's University). The accommodation and administration of the animals and the experimental protocols were conducted per the principles delineated in the Guide for the Care & Use of Lab Animals following the National Committee of Bioethics NCBE (2023). The Ethical code number 511-89.

Preparation of Gum Arabic Extract

Aqueous extracts of GA powders were prepared following the method of Todorović et al.[22] with modifications. Briefly, 20 grams of GA powder were mixed with 180 mL of deionized water and homogenized; the mixture was then agitated at 200 rpm for 1 h at room temperature. The extraction was repeated twice, and the filtrates were combined and concentrated using a rotary evaporator at 55°C under reduced pressure.

Active Compounds Profile of GA Extract Using GC-MS

GC-MS analysis was conducted on a methanol 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 0.1 min. Helium was the carrier gas at a 1 mL/min flow rate. The column temperature was programmed as follows: 50°C for 3 min, followed by a ramp of 5°C/min to 80°C, and then 10°C/min to 340°C. The inner temperature of the detector was set at 250°C and 340°C, respectively, with a solvent delay of 4 min. 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 [23].

Biological Activities of GA Extract

DPPH-scavenging Activity: The antioxidant activity of GA was evaluated as follows, 1 mL of GA (2, 4, 6, 8, and 10%) mixed with 3 mL of DPPH, maintained for 60 min, and the developed color was measured at 517 nm using a colorimeter [24]. The OD is recorded in the equation 1.

$$\%\ Antioxidant\ activity = \frac{OD\ DPPH - OD\ sample}{OD\ DPPH} \times 100$$

Antibacterial and Antifungal Activity: The bactericidal activity of GA was evaluated using a disc assay [25]. The PCA plates were inoculated with 100 µL of *Bacillus cereus* (BC), *Streptococcus aereus* (SA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP) cultures, then 8 mm discs saturated with GA concentrations were placed on the PCA surface. The plates were kept at 37°C for 24 h, and then the IZDs were recorded (mm) [26,27]. The MIC was evaluated following Saad et al.[28].

The antifungal potential of GA concentrations (2-10%) was tested against pathogenic fungi *Aspergillus niger, Fusarium oxysporuim, Penicillium chrysogenum, Alternaria alternata, Pythium aphanidermatum,* and *Podosphaera xanthii*. These pathogens were isolated from spoiled feed and confirmed through genetic analysis using 16S rRNA gene sequencing. A disc diffusion assay was employed to assess the antimicrobial activity of GA. The fungal suspensions were inoculated onto PDA plates. Sterile paper discs impregnated with various GA concentrations were placed on the inoculated plates 25°C for 7 days. After incubation, the diameter of the inhibition zones was measured (mm) [25].

Anticancer Activity: Breast cancer cell lines sourced from the American Type Culture Collection (ATCC) were initially 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 cells per milliliter using a growth medium (FBS-enriched DMEM). Subsequently, 100 µL 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 a constant temperature of 37°C for 24 h. The culture medium was then replaced with fresh medium supplemented with varying concentrations of FBS and GA (2-10%), followed by an additional 48 hours of 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 [29].

Experimental Layout

One hundred and sixty weaned male New Zealand White rabbits (age: ~35 days, average weight: 669 g) were randomly allocated to four experimental groups, each consisting of 40 rabbits and 10 replicates. The housing environment maintained a 12-h light-dark cycle, temp (36-38°C), % RH (71-75%), and THI of 36.5 *(Table 1)*. All experimental groups were under heat stress: control group fed a basal under heat stress 36.8°C; G1, rabbits treated with GA 4% in drinking water; G2, rabbits treated with GA 6% for a month; G3, rabbits treated with GA 10% for a month under heat stress.

Growth Performance

At the end of the experiment (35 days), the rabbits were euthanized by cervical dislocation. Subsequently, 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 [30].

Blood Biochemistry

Liver function: Liver enzymes, AST, and ALT were measured using kits from Spectrum, Sigma, UK. Kidney function: Urea and Creatinine levels were also determined using Spectrum kits [31,32]. Oxidative stress markers, MDA, and defense system CAT, POD, and SOD were measured using recommended kits [33,34]. Hormonal analysis: Sexual hormones were measured following the methods described by Steyn et al.[35].

Heat Stress, Proinflammatory, and Precancerous Markers

Total RNA was extracted following the method described by Dong et al.[36]. 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 2)* were employed for qRT-PCR. The 2-ΔΔCt method determined relative gene expression [37].

Table 1. *Monthly values of ambient temperature, relative humidity, and temperature-humidity index throughout the study period*

Item	July	August	Overall
AT (°C)	38±0.2	37±0.1	39.5±0.3
RH (%)	71.33±0.6	75.65±0.9	73±0.8
THI	37±0.8	36±0.7	36.5±0.2

n=3, Data are presented as mean ±SE. AT: ambient temperature, RH: relative humidity, THI: temperature-humidity index

Table 2. *Forward and reverse primers sequence for Hsp70, hsp90, Bax, Caspase 3, IL-6 genes*

Gene	Primer 5-3	MW
Hsp70	F: ATCACCATCACCAACGAC R: ACTTGTCCAGCACCTTCTT	70-kDa
hsp90	F: ATCACTGGTGAGAGCAAGAAGGC R: TTAGTCGACCTCCTCCATCTTGCT-3	90 kD
Bax	F: ACACCTGAGCTGACCTTG, R: GCCCATGATGGTTCTGATC	21.1 kDa
Caspase 3	F: CTGAACTTCGGGGTGATCG, R: GCT TGG TGG TTT GCT ACG AC	17 kDa
IL-6	F: CCACTTCACAAGTCGGAGGCTTA R: CCAGTTTGGTAGCATCCATCATTTC	25 kDa

Histology

Liver and Kidney tissue samples were fixed in 10% buffered formalin saline for histopathological analysis. Sections (0.5 cm thick) were prepared using standard paraffin-embedding techniques. Subsequently, paraffin specimens (5-µm in thick) were stained with H&E stain to assess kidney damage. After GA and cisplatin treatments, acute tubular necrosis was evaluated in the outer medulla and cortex of the kidney [38].

Statistical Analysis

A one-way ANOVA test was conducted using SPSS (version 22, USA). The triplicate data means were compared using LSD to indicate the differences were considered statistically significant at $P<0.05$.

Results

The Chemistry of Gum Arabic Extract

Active Compounds Profile: GC-MS analysis of a GA extract identified diverse bioactive compounds. *Table 3* summarizes these compounds' retention time (RT), molecular weight, peak area, and reported biological activities. The GC/MS profile of GA showed a variety of active compounds; the main compounds in the GC/MS profile were saccharides, i.e., arabinose, galactose, and rhamnose accounted for 41%, followed by phenolic acids with 36.1% (ferulic acid, caffeic acid, p-coumaric acid, syringic acid, gallic acid, ellagic acid, tannic acid, cinnamic acid, and chlorogenic acid, also essential fatty acids and flavonoids were detected in the profile.

Antioxidant Activity: Gum arabic extract, containing 66 mg/mL phenolic compounds and 24 mg/mL flavonoids, demonstrated significant DPPH free radical scavenging activity comparable with ascorbic acid (AsA) with a value of 92% *(Fig. 1)*. Notably, a 10% (w/v) concentration of GA exhibited a potent scavenging effect, neutralizing 91% of DPPH radicals with an IC50 of 2%

Table 3. GC-MS profile of active compounds in gum arabic extract

RT (min)	Compound	Molecular Weight (g/mol)	Chemical Formula	% Area
5.23	Arabinose	150.13	$C_5H_{10}O_5$	12.5±0.9
7.45	Galactose	180.16	$C_6H_{12}O_6$	18.3±0.8
9.12	Rhamnose	164.16	$C_6H_{12}O_5$	8.7±0.2
11.34	Glucuronic Acid	194.14	$C_6H_{10}O_7$	10.2±0.1
13.56	4-Hydroxybenzoic Acid	138.12	$C_7H_6O_3$	5.6±0.3
15.78	Catechol	110.11	$C_6H_6O_2$	3.4±0.1
17.89	Vanillin	152.15	$C_8H_8O_3$	7.8±0.6
20.12	Ferulic Acid	194.18	$C_{10}H_{10}O_4$	9.1±0.2
22.34	Quercetin	302.24	$C_{15}H_{10}O_7$	6.3±0.3
24.56	β-Sitosterol	414.72	$C_{29}H_{50}O$	4.5±0.1
26.78	Oleanolic Acid	456.70	$C_{30}H_{48}O_3$	3.2±0.2
28.90	Linoleic Acid	280.45	$C_{18}H_{32}O_2$	2.8±0.3
30.12	Palmitic Acid	256.43	$C_{16}H_{32}O_2$	4.1±0.2
32.34	Stearic Acid	284.48	$C_{18}H_{36}O_2$	3.7±0.6
34.56	Caffeic Acid	180.16	$C_9H_8O_4$	5.9±0.3
36.78	p-Coumaric Acid	164.16	$C_9H_8O_3$	4.8±0.2
38.90	Syringic Acid	198.17	$C_9H_{10}O_5$	3.5±0.3
40.12	Luteolin	286.24	$C_{15}H_{10}O_6$	2.9±0.1
42.34	Apigenin	270.24	$C_{15}H_{10}O_5$	2.6±0.2
44.56	Kaempferol	286.24	$C_{15}H_{10}O_6$	3.1±0.3
46.78	Myricetin	318.24	$C_{15}H_{10}O_8$	2.4±0.2
48.90	Gallic Acid	170.12	$C_7H_6O_5$	4.3±0.1
50.12	Ellagic Acid	302.19	$C_{14}H_6O_8$	3.8±0.2
52.34	Tannic Acid	1701.20	$C_{76}H_{52}O_{46}$	1.9±0.2
54.56	Cinnamic Acid	148.16	$C_9H_8O_2$	2.7±0.1
56.78	Chlorogenic Acid	354.31	$C_{16}H_{18}O_9$	3.6±0.2
58.90	Rutin	610.52	$C_{27}H_{30}O_{16}$	2.1±0.3
60.12	Hesperidin	610.56	$C_{28}H_{34}O_{15}$	1.8±0.4

n=3, Data are presented as mean ±SE. retention time (RT)

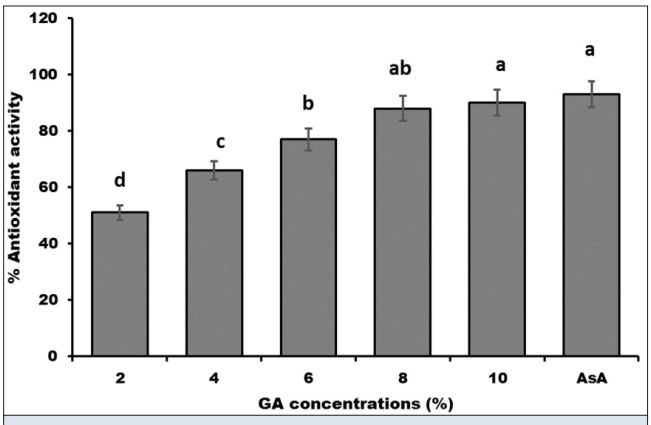

Fig 1. Antioxidant activity of gum arabic extract against DPPH radicals. *n*=3, Data are presented as mean ±SE. Lowercase letters above columns indicate significant differences (P<0.05)

Antimicrobial Activity: *Table 4* shows the antibacterial activity of GA at different concentrations, where GA 10% reduced the growth of pathogenic bacteria with IZDs of 12-30 mm; s*taphylococcus aureus* was the most vulnerable bacteria to GA; however, *Klebsiella pneumonia was the most* resistant. This study evaluated the antifungal efficacy of GA against six pathogenic microorganisms. Inhibition zone diameters (IZDs) around GA-treated discs increased proportionally with GA concentration, ranging from 13 to 31 mm *(Table 4, Fig 2)*. The 10% GA concentration exhibited the strongest antimicrobial activity. Among the tested fungi, *P. chrysogenum, F. oxysporuim, A. alternata,* and *B. cinerea* showed the highest resistance, displaying smaller inhibition zones. At the highest GA concentration, IZDs ranged between 27 and 31 mm

Table 4. *Antifungal activity of gum arabic extract (2-10 %) against pathogenic microorganisms*

Microrganisms		GA (%)/IZD (mm)				
		2	4	6	8	10
Pathogenic bacteria	*Bacillus cereus*	13±0.2b	16±0.6b	19±0.5b	21±0.6b	27±0.5b
	Staphylococcus aureus	16±0.3a	20±0.2a	24±0.3a	26±0.5a	30±0.7a
	Escherichia coli	12±0.8bc	15±0.5bc	18±0.5bc	22±0.8bc	25±0.2c
	Klebsiella pneumonia	10±0.2c	14±0.6c	16±0.8c	17±0.2c	21±0.3d
Pathogenic fungi	*F. oxysporuim*	14±0.2b	21±0.5b	25±0.3b	28±0.3b	32±0.2b
	A. niger	13±0.3c	18±0.4c	22±0.2c	26±0.3bc	28±0.8c
	P. chrysogenum	16±0.3b	18±0.3c	24±0.3b	27±0.2bc	32±0.3bc
	Alternaria alternata	19±0.5a	23±0.1a	27±0.1a	30±0.3a	35±0.5a
	P. aphanidermatum	15±0.6bc	16±0.3d	21±0.5d	22±0.1c	27±0.7c
	Podosphaera xanthii	14±0.5bc	18±0.6c	22±0.6c	25±0.5bc	28±0.9bc

n=3, Data are presented as mean ±SE. Different lowercase letters in the same column indicate significant differences (P<0.05)

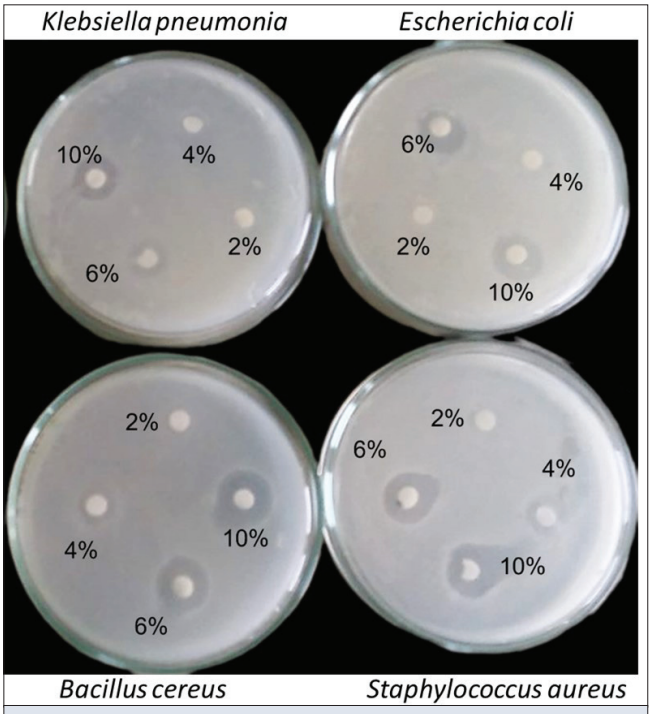

Fig 2. Antimicrobial activity of gum arabic against selected bacterial strains (disc assay)

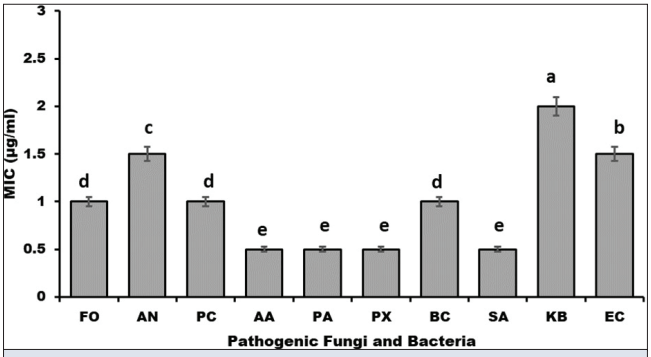

Fig 3. MIC values of gum arabic extract against pathogenic bacteria and fungi. *n*=3, Data are presented as mean ±SE. Lowercase letters above columns indicate significant differences (P<0.05). *Fusarium oxysporium* (FO), *Aspergillus niger* (AN), *P. chrysogenum* (PC), *Alternara alternara* (AA), *P. aphanidermatum* (PA), *Podosphaera xanthii* (PX), *Bacillus cereus* (BC), *Staphylococcus aereus* (SA), *Klebsila pneumonia* (KP), *Esherchia coli* (EC)

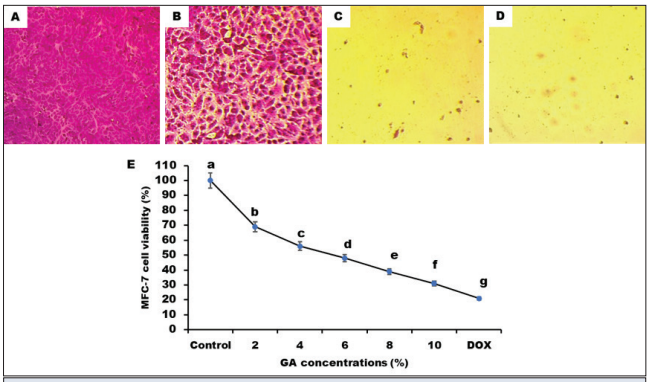

Fig 4. (A) Microscopic images of the cytotoxicity effect of MCF-7 viability GA on the. (B) photograph of the cytotoxicity effect of GA 2% on the MCF-7 viability, (C) the cytotoxicity effect of GA 10% on the MCF-7 viability compared to doxorubicin (DOX, 300 µg/mL) (D). (E) Histogram of the effect of GA concentrations on the viability of MCF-7 compared to DOX. *n*=3, Data are presented as mean ±SE. The Lowercase letters above columns indicate significant differences (P<0.05)

(Table 4). GA demonstrated significant antifungal activity, effectively inhibiting microbial growth within a 0.5-2% concentration range. *Fusarium oxysporium* and *Botrytis cinerea* exhibited the highest resistance to GA with a minimum inhibitory concentration (MIC) of 2%, while *Aspergillus niger* and *Rhizoctonia solani* were the most sensitive with an MIC of 0.5% *(Fig. 3)*.

Anticancer Activity: *Fig. 4* shows that GA concentrations had considerable anticancer activity against MCF-7 cancer cell viability. GA 10% reduced 78% of cancerous cell progress compared to doxorubicin, which inhibited MCF-7 by 81%.

Growth Performance

Table 5 presents the growth performance of rabbits under different treatments, including a control group and three groups receiving GA at varying concentrations (4%, 6%, and 10%). The parameters measured include: Live body weight (LBW) at 5 weeks, body weight gain (BWG) from 5 to 13 weeks, feed intake (FI) from 5 to 13 weeks, feed conversion ratio (FCR) from 5 to 13 weeks, growth rate (GR) from 5 to 13 weeks, and performance index (PI). The GA treatments, particularly at higher concentrations (6% and 10%), showed significant improvements in growth parameters compared to the control group.

The results showed that GA 6% and 10% groups exhibited significantly higher BWG compared to the control. GA 6% showed a 12.2% increase, and GA 10% showed a 19.6% increase. Similar to BWG, GA 6% and 10% groups had significantly higher GR than the control. GA 6% showed a 11.4% increase, and GA 10% showed a 18.8% increase. Also, the 6% and 10% groups displayed significantly better FCR than the control, indicating improved feed efficiency. GA 6% showed a 7.1% improvement, and GA 10% showed a 9.7% improvement. The performance index of GA 6% and 10% groups had significantly higher PI values than the control, suggesting better overall performance. GA 6% showed a 10.6% increase, and GA 10% showed a 14.3% increase.

The improved growth performance in the GA groups could be attributed to various factors, i.e., improved feed digestibility and nutrient absorption, where GA might enhance the digestibility of nutrients in the feed, leading to better nutrient utilization and growth. Reduced oxidative stress, where GA has antioxidant properties that could help mitigate the negative effects of heat stress on oxidative stress, leading to improved growth and improved gut health, where GA might have beneficial effects on gut microbiota, promoting better nutrient absorption and overall health.

The results suggest that incorporating GA into the diet of rabbits under heat stress can significantly enhance their growth performance. The higher concentrations (6% and 10%) of GA appear more effective in improving BWG, GR, FCR, and PI than the control.

Blood Biochemistry

Table 6 shows that GA treatments significantly increased the blood parameters, RBCs, Hemoglobin, Hematocrit, MCH, MCV, MCHC compared to the control group ($P<0.05$). This suggests that GA supplementation improved red blood cell production and oxygen-carrying capacity, which benefit overall health and performance. Additionally, GA treatments significantly enhanced the Albumin, Total protein, and Globulin content compared to the control group ($P<0.05$) under heat stress. This indicates that GA supplementation may have improved protein synthesis and liver function under heat stress.

The liver and kidney parameters, ALT, AST, Creatinine, Urea, Triglycerides, Glucose, TC, LDL were significantly improved by GA treatments, where these parameters decreased compared to the control group ($P<0.0001$). This suggests that GA supplementation may have improved liver function, reduced oxidative stress, and improved lipid metabolism. On the other hand, GA treatments significantly increased HDL cholesterol compared to the control group ($P<0.0001$). This is beneficial as HDL cholesterol helps remove excess cholesterol from the bloodstream.

Based on the overall improvements in blood parameters, the GA 10% group appears to be the most beneficial. It consistently showed significant improvements in hematology and serum metabolites compared to the control group. However, it is important to note that further research is needed to determine the optimal GA concentration and supplementation strategy for different breeds and environmental conditions. GA 6% and GA 4% groups also showed significant improvements in most parameters compared to the control group. However, the magnitude of improvement was generally lower than in the GA 10% group. In some cases, the GA 10% group showed greater improvements than the GA 6% and GA

Table 5. *The effect of dietary GA with different concentrations on the growth performance of heat-stressed rabbits*

Treatments (mg/kg)	LBW (g)		BWG (g)	FI (g)	FCR	GR	PI
	5w	13 w	5w-13w	5w-13w	5w-13w	5w-13w	5w-13w
Control	670.14±11.3	1950±3.6c	1279.86±11.2d	4508±11.2c	3.52±0.2a	171.33±1.1d	363.36±10.3d
GA 4 %	668.33±10.3	2055±1.2b	1386.67±10.3c	4585±12.5c	3.31±0.3b	185.65±1.5c	419.37±9.5c
GA 6 %	671.36±12.5	2096±3.5b	1424.64±9.5b	4606±9.2b	3.23±0.2b	191.22±2.1b	440.64±8.2b
GA 10 %	672.58±11.6	2185±5.4a	1512.42±8.9a	4809±9.4a	3.18±0.4c	203.54±2.3a	475.65±7.7a
p-value	0.669	0.00012	0.0011	0.0013	0.00	0.0036	0.0002

n=3, Data are presented as mean ±SE. The row's lowercase letters indicate significant differences (P<0.05). live body weight (LBW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), growth rate (GR), performance index (PI)

4% groups, suggesting a possible dose-dependent effect of GA. The results suggest that GA supplementation, particularly at 10% concentration, can significantly enhance rabbits' blood hematology and chemistry under heat stress by improving red blood cell production, liver function, lipid metabolism, and antioxidant status.

The results of a study investigating the effect of different GA concentrations (0%, 4%, 6%, and 10%) on rabbits' oxidative status, liver oxidative status, and immunity parameters under heat stress are presented in *Table 6*. GA treatments 6 and 10% significantly increased TAC, SOD, and GSH-Px in serum compared to the control group (P<0.05). This suggests that GA supplementation enhanced the antioxidant defense system in rabbits while decreasing the MDA levels compared to the control group (P<0.0001). This indicates that GA supplementation reduced lipid peroxidation and oxidative damage. Similarly, the 6 and 10% GA treatments significantly increased liver TAC compared to the control group (P<0.00236). This suggests that GA supplementation improved the liver's antioxidant capacity. However, liver MDA levels were decreased compared to the control group (P<0.0001). This indicates that GA supplementation reduced oxidative damage in the liver.

The GA treatments 6 and 10% significantly increased all three immunoglobulin levels compared to the control

Table 6. *The effect of dietary GA with different concentrations on blood hematology and chemistry of heat-stressed rabbits*

Blood Parameters			**Control**	**GA 4 %**	**GA 6 %**	**GA 10 %**	**P-value**
Haematology	Hemoglobin (g/dL)		10.93±0.2c	11.69±0.5b	12.24±0.8a	11.88±0.5b	0.0122
	RBCs (x 10^6/mm3)		4.687±0.1c	5.96±0.6b	6.51±0.7a	6.15±0.5a	0.036
	WBCs (x 10^3/mm3)		5.657±0.3c	6.87±0.5b	7.42±0.6a	7.11±0.2a	0.024
	Platelet (x 10^3/mm3)		281.45±11.3	287.73±1.4	288.28±1.5	287.11±10.3	0.78
	Hematocrit (%)		28.95±0.6b	29.55±0.9ab	30.1±0.3a	29.55±0.6ab	0.041
	MCH (pg)		23.97±0.6b	25.51±1.7ab	26.06±0.8a	25.70±0.5a	0.0356
	MCV (µm^3)		67.37±0.9a	65.47±0.5b	66.02±0.6ab	65.66±0.2b	0.0123
	MCHC (g/dl)		34.22±0.8b	34.98±0.8b	35.53±0.5a	35.17±0.6a	0.023
Serum metabolites	Albumin, g/dL		3.5±0.2b	4.06±0.2ab	4.39±0.4a	4.3±0.2a	0.02
	Total protein, g/dL		5.97±0.5c	6.87±0.3b	7.33±0.1a	7.24±0.6a	0.00253
	Globulin, g/dL		2.72±0.1c	2.97±0.1a	2.97±0.2a	2.88±0.2b	0.77
	ALT, IU/L		62.63±1.3a	45.42±0.3b	39.6±0.4c	41.3±0.6b	0.0001
	AST, IU/L		32.3±0.8a	27.7±0.5b	22.3±0.5c	21.3±0.8c	0.0001
	Creatinine, mg/dL		1.25±0.2a	0.99±0.9b	1.01±0.1ab	0.92±0.01b	0.036
	Urea, mg/dL		40.01±0.5a	39.22±0.4a	36.55±0.2b	36.46±0.5b	0.01
	LDL, mg/dL		34.69±0.6a	24.46±0.3b	21.33±0.5c	22.3±0.2c	<0.0001
	Triglycerides, mg/dL		86.68±1.1a	68.41±0.1b	55.36±0.3d	59.33±0.6c	<0.0001
	Glucose, mg/dL		84.18±1.2a	83.53±0.6a	79.66±0.8b	79.57±0.6b	0.032
	TC, mg/dL		91.01±0.9	77.95±1.2b	72.08±0.9b	73.66±0.3b	0.012
	HDL, mg/dL		37.34±0.5b	39.07±0.8b	41.13±0.5a	41.04±0.5a	0.029
Oxidative status	Serum	TAC, ng/mL	0.28±0.02c	0.46±0.05b	0.63±0.02a	0.59±0.01ab	0.0112
		SOD, U/mL	0.36±0.01c	0.52±0.06a	0.54±0.03a	0.49±0.06b	0.041
		Glutathione, mg/dL	14.14±0.2	14.97±0.5	14.81±1.2	14.69±0.8	0.89
		GSH-Px, mg/dL	3.03±0.3c	5.23±0.3a	4.89±0.3b	4.77±0.2b	0.011
		MDA, nmol/mL	13.42±0.6a	8.32±0.5b	8.26±0.5b	8.14±0.3b	0.033
	Liver	TAC, nmol/mL	0.86±0.02b	1±0.1a	1.15±0.2a	1.05±0.2a	0.0236
		MDA, nmol/mL	6.22±0.6a	4.79±0.b6	3.42±0.6c	3.5±0.2c	<0.0001
Immunity	IgG, mg/dL		489.33±12.3b	523.99±11.3a	529.19±11.6a	525.3±13.8a	0.0223
	IgA, mg/dL		134.75±14.3b	162.43±10.3a	163.75±6.3a	161.3±12.6a	0.0132
	IgM, mg/dL		115.87±10.3c	140.62±14.5ab	144.1±9.2a	139.5±11.7b	<0.0001

n=3, Data are presented as mean ±SE. The row's lowercase letters indicate significant differences (P<0.05). Red blood cells (RBCs), white blood cells (WBCs), Mean corpuscular Hemoglobin (MCH), Mean corpuscular volume (MCV), Mean corpuscular Hemoglobin concentration (MCHC), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total antioxidant content (TAC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), immunoglobin G (IgG), *immunoglobin A* (IgA), *immunoglobin M* (IgM)

group (P<0.0001). This suggests that GA supplementation enhanced the immune response in rabbits. The results suggest that GA supplementation at 6 or 10% can significantly improve rabbits' oxidative status, liver oxidative status, and immune function under heat stress.

The Expression of Inflammatory, Heat Stress Protein and Cancer Markers

Table 7 shows the gene expression levels of various markers in rats exposed to heat stress and fed different concentrations of GA extract (0%, 4%, 6%, and 10%). Proinflammatory Cytokines: GA 6% showed the lowest expression in IL-1β (1.4d), followed by GA 10% (2.1c) and GA 4% (4.0b). The control group had the highest expression (9.7a), with a relative decrease of 85% compared to the control. GA 10% showed a 78% reduction, and GA 4% showed a 59% reduction. Also, the expression of TNF-α reduced in GA 6% showed a decrease of 87% compared to the control. GA 10% showed an 82% reduction, and GA 4% showed a 64% reduction.

The heat stress proteins: All GA groups showed significantly higher expression of HSP70 and HSP90 than the control. GA 6% had the highest expression (45a), followed by GA 10% (44a) and GA 4% (41a). Relative increases of 273% in GA 4%, 309% in GA 6%, and GA 10% showed a 291% increase. The increases in HSP90 expression ranged between 130-210%.

Precancerous markers, BAX and Casp-3, showed the lowest expression (1.3d, 1.3c) in GA 6%, followed by GA 10% (2.2c, 1.5c) and GA 4% (5.0b, 3.2b). The control group had the highest expression (10a, 7.2a).

GA supplementation, especially at lower concentrations (6% and 10%), significantly reduced the expression of proinflammatory cytokines (IL-1β and TNF-α) and precancerous markers (BAX and Casp-3) in heat-stressed rabbits while considerably increasing the expression of heat stress proteins (HSP70 and HSP90), which are essential for cellular protection against stress.

Histology of Liver and Kidney of Heat-Stressed Rabbits

Fig. 5-I showed the histological examination of the liver revealed the following: (A) Control heat-stressed rabbits exhibited hepatic damage characterized by necrosis, inflammation, bile duct proliferation, and cholestasis. (B) In rabbits treated with 4% GA, signs of regeneration were observed in the hepatocytes with mild inflammation. (C) The 6% GA group displayed normal liver architecture with proper arrangement of hepatocytes, bile ducts, and sinusoids. (D) The 10% GA group also exhibited a normal arrangement of hepatocytes with normal sinusoidal dilation.

Histological examination of the kidney presented in *Fig. 5-II* where (A) in control heat-stressed rabbits, proximal convoluted tubules displayed cuboidal epithelial cell necrosis and exfoliation of the brush border. (B) In the GA 4% group, kidney sections showed necrosis, epithelial cell swelling, and tubules with exfoliated brush borders. (C) The 6% GA group under heat stress exhibited normal kidney parenchyma with no significant changes. (D) Histological examination of the 10% GA group revealed normal parenchyma with intact glomeruli and tubules.

Gut Microbiota

Fig. 6 presents the gut microbial count (CFU/g) of different bacterial groups in the digestive tract of heat-stressed rabbits treated with various GA concentrations. The results suggest that GA supplementation, particularly at higher concentrations (6% and 10%), significantly reduced the microbial counts of most bacterial groups compared to the control group. The treatments GA 6% and GA 10% showed a significant decrease (25-40%) in TBC, coliform, *E. coli*, *Salmonella* compared to the control. GA 4% showed a moderate decrease. While GA 6% and GA 10% showed a significant increase (45%) in LAB counts compared to the control. GA 4% showed a moderate increase.

The reduction in TBC, *E. coli*, Coliform, and *Salmonella* counts suggests that GA supplementation may help

Table 7. *The effect of dietary treatments GA extract on proinflammatory cytokines, precancerous markers, and heat stress protein in heat stressed rats*

Genes	Proinflammatory Markers		Heat Stress Protein		Precancerous Markers	
	IL-1β	TNF-α	HSP70	HSP90	BAX	Casp-3
Control	9.7±0.3a	9.6±0.6a	11±0.6b	10±0.3c	10±0.3a	7.2±0.6a
GA 4%	4.0±0.2b	3.5±0.5b	44±0.9a	31±0.5a	5.0±0.4b	3.2±0.2b
GA 6%	1.4±0.1d	1.2±0.2c	41±1.1a	23±0.8b	1.3±0.5d	1.3±0.1c
GA 10%	2.1±0.6c	1.7±0.1c	45±1.2a	29±0.5ab	2.2±0.7c	1.5±0.2c
P value	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001

n=3, Data are presented as mean ±SE. The column's lowercase letters indicate significant differences (P<0.05)

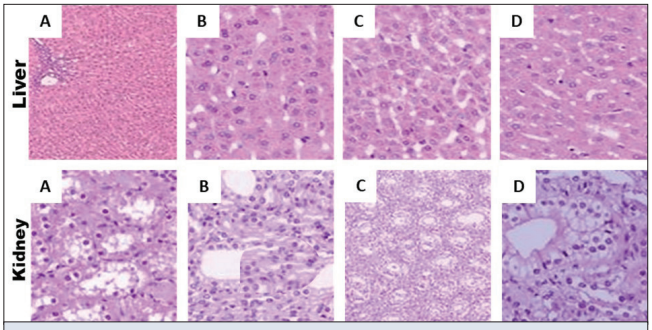

Fig 5. Histopathological evaluation of liver and kidney sections in paracetamol-induced hepatotoxicity in rabbits treated with GA. (H&E staining, 40x, scale bar = 100 µm, n = 6 rabbits/group). A) untreated control under heat stress, B) GA 4%-treated group under heat stress, C) GA 6%-treated group

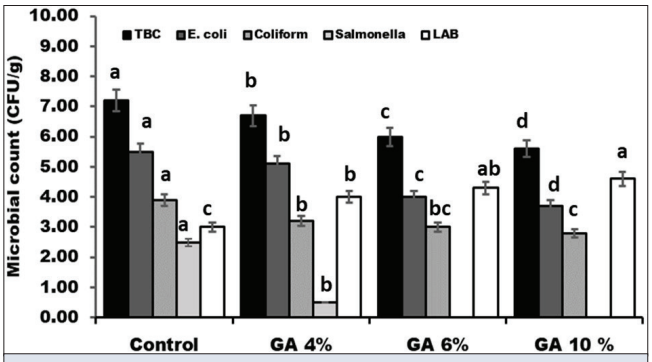

Fig 6. The effect of dietary GA on the gut microbiota (total bacterial count, *E. coli* count, Coliform, Salmonella, and Lactic acid bacteria count) of heat-stressed rabbits. *n*=3, Data are presented as mean ±SE. Lowercase letters above columns indicate significant differences (P<0.05)

to improve gut health and reduce the risk of bacterial infections in heat-stressed rabbits. The increase in LAB counts is beneficial as LAB are beneficial bacteria that contribute to gut health and digestion.

Discussion

The detrimental effects of heat stress on rabbit production are a growing concern, demanding innovative and sustainable solutions. Gum arabic, a natural polysaccharide with multifaceted biological activities, has emerged as a promising feed additive to mitigate these challenges [39]. The efficacy of GA in alleviating heat stress is attributed to its complex composition and diverse mechanisms of action. GA's high molecular weight and branched structure contribute to its prebiotic properties, fostering the growth of beneficial gut bacteria [40]. Its phenolic compounds and polysaccharides act as potent antioxidants, scavenging free radicals and enhancing antioxidant enzyme activity [41]. Furthermore, GA can modulate immune responses and reduce inflammation, contributing to overall health and resilience [42].

These mechanisms are interconnected, creating a synergistic effect that enhances the overall well-being of heat-stressed rabbits. For instance, the prebiotic effect of GA promotes a healthy gut microbiota, which in turn improves nutrient absorption and reduces inflammation. This improved gut health contributes to enhanced growth performance and reduced oxidative stress. Similarly, the antioxidant properties of GA protect cellular components from oxidative damage, preserving organ function and improving blood parameters [43,44].

Heat stress induces a cascade of physiological changes that negatively impact performance of animal growth [45]. Reduced feed intake is a primary consequence of hormonal imbalances and increased energy expenditure for thermoregulation [46]. GA can counteract this by modulating gut hormones such as ghrelin and leptin, potentially stimulating appetite and improving feed intake. Moreover, GA enhances fiber digestion and nutrient absorption by promoting the growth of cellulolytic and saccharolytic bacteria in the gut. This improved nutrient utilization translates to better feed conversion efficiency and enhanced growth rate. Furthermore, GA can reduce the inflammatory responses associated with heat stress. Chronic inflammation can impair nutrient utilization and growth performance. GA helps maintain a healthy gut environment and optimize nutrient utilization by modulating immune responses and reducing the production of pro-inflammatory cytokines [47]. El-Sabrout et al.[48] demonstrated that GA supplementation at 1.5% of the diet significantly improved body weight gain and feed conversion ratio in heat-stressed rabbits. The study attributed these effects to enhanced gut health and nutrient absorption.

Heat stress disrupts the delicate balance of blood parameters, leading to alterations in hematological and biochemical profiles [49]. Elevated cortisol levels, a hallmark of stress, can impair immune function and metabolic processes [50]. GA has demonstrated the ability to reduce cortisol levels, mitigating stress responses. This reduction in cortisol may be mediated by the modulation of the hypothalamic-pituitary-adrenal (HPA) axis.

Changes in lipid profiles, such as increased cholesterol and triglyceride levels, are also observed in heat-stressed animals. GA can improve lipid profiles by binding bile acids and promoting their excretion, thereby reducing cholesterol and triglyceride levels [51]. Furthermore, GA can enhance glucose metabolism by improving insulin sensitivity and reducing insulin resistance [52]. Hematological parameters, such as red blood cell count, hemoglobin concentration, and white blood cell count, are also affected by heat stress [53]. GA can positively influence these parameters, reflecting improved overall health status. This improvement may be attributed to the antioxidant and immunomodulatory properties of GA, which protect blood cells from oxidative damage and

enhance immune function. GA reduces serum levels of stress markers such as cortisol and heat shock proteins (HSPs) while improving liver and kidney function markers (e.g., ALT, AST, creatinine) [54].

Heat stress induces a surge in ROS production, overwhelming the antioxidant defense system and leading to oxidative stress [55]. This imbalance can damage cellular components, including lipids, proteins, and DNA, impairing physiological functions. GA's potent antioxidant properties are crucial in mitigating this oxidative damage. GA's phenolic compounds and polysaccharides act as free radical scavengers, neutralizing ROS and preventing cellular damage [56]. Furthermore, GA can enhance the activity of antioxidant enzymes such as SOD, CAT, and GPx, bolstering the antioxidant defense system [57]. These enzymes play crucial roles in detoxifying ROS and maintaining cellular redox balance. Furthermore, GA protects cellular membranes and other vital components from oxidative damage by reducing MDA levels, a marker of lipid peroxidation, and enhancing antioxidant enzyme activity [51]. This improvement in oxidative status contributes to heat-stressed rabbits' overall health and well-being.

The gut microbiota is critical in nutrient digestion, immune modulation, and overall health [58]. Heat stress can disrupt the delicate balance of gut microbiota, leading to dysbiosis and impaired gut function [59]. GA acts as a prebiotic, selectively promoting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* [60]. These bacteria produce short-chain fatty acids (SCFAs), which serve as energy sources for intestinal epithelial cells, enhance gut barrier function, and modulate immune responses. By inhibiting the growth of pathogenic bacteria, GA helps maintain a healthy gut environment and prevent gut dysbiosis. This modulation of gut microbiota enhances gut barrier function, improving nutrient absorption and reducing inflammation. A healthy gut barrier prevents the leakage of endotoxins and other harmful substances into the bloodstream, reducing systemic inflammation.

Heat stress can induce pathological changes in various organs, including the liver, kidneys, and intestines. GA has demonstrated protective effects on organ histology by mitigating the adverse effects of heat stress. GA can reduce hepatic lipid accumulation in the liver, a common consequence of heat stress. This reduction may be attributed to the ability of GA to modulate lipid metabolism and enhance antioxidant defense. GA can improve tubular integrity in the kidneys, protecting against heat stress-induced renal damage. The antioxidant and anti-inflammatory properties of GA may mediate this protective effect. GA can enhance villus height and crypt depth in the intestines, improving nutrient absorption and gut barrier function. This improvement may be attributed to the prebiotic effect of GA, which promotes the growth of beneficial bacteria and enhances gut health. While the existing research highlights the potential of GA as a feed additive for heat-stressed rabbits, further studies are needed to optimize its application and explore its long-term effects. Future research should focus on determining the optimal dosage of GA for different breeds and ages of rabbits under varying heat stress conditions, investigating the mechanisms by which GA exerts its beneficial effects, including its interactions with gut hormones, immune cells, and signaling pathways. Also, assessing the long-term effects of GA supplementation on growth performance, reproductive performance, and overall health of rabbits. Exploring the synergistic effects of GA with other feed additives, such as probiotics, prebiotics, and antioxidants. Evaluating the economic feasibility of GA supplementation in rabbit production. Utilizing advanced omics technologies to understand the changes produced by GA comprehensively. Furthermore, it is essential to consider the source and quality of GA, as variations in composition and purity can affect its efficacy. Standardization of GA production and quality control are crucial for ensuring consistent results. In conclusion, GA holds excellent promise as a natural feed additive for mitigating the adverse effects of heat stress in rabbits. Its multifaceted biological activities, including prebiotic, antioxidant, and immunomodulatory properties, enhance growth performance, improve blood parameters, reduce oxidative stress, modulate gut microbiota, and protect organ histology. Further research is warranted to optimize its application and explore its full potential in rabbit production.

Declaration

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical Approval: The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory

Animals (2022). Ethical code number ZU-IACUC/2/F/394/2022. Written informed consent was obtained from the owners for the participation of their animals in this study.

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