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Nephroprotective effects of *Acacia senegal* against aflatoxicosis via targeting inflammatory and apoptotic signaling pathways

Obeid Shanab [a], Samir M. El-Rayes [b], Waleed F. Khalil [c], Noha Ahmed [b], Afaf Abdelkader [d, *], Nashwa H. Aborayah [e, f], Ahmed M. Atwa [g], Faten I. Mohammed [h], Hend E. Nasr [i], Samah F. Ibrahim [j], Amr M. Khattab [k, l], Mohammed Alsieni [m], Ali Behairy [e], Liana Fericean [n, *], Lina A. Mohammed [i], Ahmed Abdeen [°o, *]

[a] *Department of Biochemistry, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt*
[b] *Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt*
[c] *Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt*
[d] *Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Benha University, Benha 13518, Egypt*
[e] *Department of Pharmacology, Faculty of Medicine, Benha University, Benha 13518, Egypt*
[f] *Department of Pharmacology, Faculty of Medicine, Mutah University, Mutah 61710, Jordan*
[g] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Cairo 11829, Egypt*
[h] *Department of Physiology, Faculty of Medicine for Girls, Al-Azhar University, Cairo 11884, Egypt*
[i] *Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Benha University, Benha 13518, Egypt*
[j] *Department of Clinical Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia*
[k] *Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University, Cairo 11956, Egypt*
[l] *Department of Clinical Toxicology, Dammam Poison Control Center, MOH, Dammam 32245, Saudi Arabia*
[m] *Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah 22254, Saudi Arabia*
[n] *Department of Biology and Plant Protection, Faculty of Agriculture, University of Life Sciences "King Michael I" from Timișoara, Calea Aradului 119, CUI 3487181, Romania*
[°o] *Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt*

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ABSTRACT

Aflatoxin B_1 (AFB1) is a common environmental pollutant that poses a major hazard to both humans and animals. *Acacia senegal* (Gum) is well-known for having antioxidant and anti-inflammatory bioactive compounds. Our study aimed to scout the nephroprotective effects of Acacia gum (Gum) against AFB1-induced renal damage. Four groups of rats were designed: Control, Gum (7.5 mg/kg), AFB1 (200 μg/kg b.w) and AFB1-Gum, rats were co-treated with both Gum and AFB1. Gas chromatography-mass spectrometry (GC/MS) analysis was done to determine the phytochemical constituents in Gum. AFB1 triggered profound alterations in kidney function parameters (urea, creatinine, uric acid, and alkaline phosphatase) and renal histological architecture. Additionally, AFB1 exposure evoked up-regulation of mRNA expression levels of inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor α (TNFα), inducible nitric oxide synthase (iNOS), and nuclear factor kB p65 (NF-κB/P65) in renal tissue. The oxidative distress and apoptotic cascade are also instigated by AFB1 intoxication as depicted in down-regulated protein expression of the nuclear factor erythroid 2–related factor 2 (Nrf2) and superoxide dismutase type 1 (SOD1) along with upregulation of cytochrome c (Cyto c), and cleaved Caspase3 (Casp3–17 and 19) in renal tissue. In conclusion, current study obviously confirms the alleviating effects of Gum

Abbreviations: AFB1, aflatoxin B_1; AFO, aflatoxin-exo-8,9-epoxide; Gum, *Acacia senegal*; ALP, alkaline phosphatase; Cl. Casp3–17/19, cleaved caspase3–17/19; Cyto c, cytochrome c; GC/MS, Gas chromatography-mass spectrometry; GSH, glutathione; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; NF-κB/P65, nuclear factor kappa-B transcription factor/P65; Nrf2, nuclear factor erythroid2–related factor2; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; TNF-α, tumor necrosis factor-α

* Corresponding authors.
E-mail addresses: shanab.bio@vet.svu.edu.eg (O. Shanab), Samir_elrayes@yahoo.com (S.M. El-Rayes), wk@vet.suez.edu.eg (W.F. Khalil), nohaahmed2552015@gmail.com (N. Ahmed), afaf.abdelkader@fmed.bu.edu.eg (A. Abdelkader), nashwa.aborayah@fmed.bu.edu.eg (N.H. Aborayah), ahmed-atwa@eru.edu.eg (A.M. Atwa), fatenebrahem.medg@azhar.edu.eg (F.I. Mohammed), hend.mosalm@fmed.bu.edu.eg (H.E. Nasr), sfibrahim@pnu.edu.sa (S.F. Ibrahim), ammkhattab@moh.gov.sa (A.M. Khattab), malsieni@kau.edu.sa (M. Alsieni), ali.behery@fmed.bu.edu.eg (A. Behairy), mihaelafericean@usab-tm.ro (L. Fericean), lina.mohammed@fmed.bu.edu.eg (L.A. Mohammed), ahmed.abdeen@fvtm.bu.edu.eg (A. Abdeen).

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supplementation against AFB1-induced renal dysfunction, oxidative harm, inflammation, and cell death. These mitigating effects are suggested to be attributed to Gum's antioxidant and anti-inflammatory activities. Our results recommend Gum supplementation as add-on agents to food that might aid in protection from AFB1-induced nephrotoxicity.

1. Introduction

Aflatoxins are secondary metabolites produced by certain *Aspergillus* fungi (*Aspergillus flavus* and *A. parasiticus*), and possess a bisdifuran ring structure (Gao et al., 2021). They are inescapable environmental contaminants due to their pervasiveness in the food chain and can cause serious health consequences. Among them, aflatoxin B_1 (AFB1) is the most ubiquitous poisonous one and exhibits immunosuppressive, neurotoxic, reprotoxic, teratogenic, genotoxic, and carcinogenic activities and is designated as a class 1 carcinogen (Yilmaz and Bag, 2022; Zhang et al., 2022; Wang et al., 2022; Karamkhani et al., 2020).

The toxicity of AFB1 is mainly owing to its biotransformation to AFB1-exo-8,9-epoxide (AFBO) via hepatic cytochrome-P450. AFBO is a highly reactive toxic intermediate that attacks the guanine residues of DNA, creating adducts that lead to well-renowned mutations (Wang et al., 2022). Subsequently, AFBO is detoxified by phase 2 glutathione (GSH) system to less toxic GSH-complex that is ready to excreted from cells via the mercapturic acid pathway (Dlamini et al., 2021). It is noteworthy that in addition to the liver, the kidney is a potential target since AFB1. AFBO are selectively absorbed and concentrated by tubular cells and accumulated in the renal medulla prior to being excreted in the urine and causing direct toxic damage to renal tubules (Wang et al., 2022).

There is a plethora of evidence indicating that oxidative stress and apoptotic pathway are the pathological foundation of AFB1-prompted renal damage. Depletion of antioxidant enzymes as well as formation of reactive oxygen species (ROS), provoking tissue injury such as DNA adducts, mitochondrial perturbation, protein misfolding, and eventually necroptosis (Dlamini et al., 2021; Meki et al., 2001; Owumi et al., 2020; Li et al., 2022). Therefore, antioxidants supplementation could be a feasible therapeutic strategy to halt AFB1 -induced renal damage.

Over the last few decades, considerable studies have recognized plant-based natural ingredients as potential sources of drugs, including *Acacia senegal* (Gum). Gum is a natural emulsifier exuded by incision of the branches and stems of *A. senegal* tree (Ashour et al., 2022). It has been certified by the Food and Drug Administration (FDA) procuration as a safe dietary fiber (Hassanien, 2019; Elshama, 2018). It comprises arabinogalactan polysaccharides, proteins, and minerals (Abdin et al., 2022). Thus, Gum is exploited in medicinal activities because of its therapeutic potential owing to its antioxidant (Hassanien, 2019), immune-modulatory (Ali et al., 2020), anti-inflammatory (Elshama, 2018), and antibacterial activities (Elshama, 2018). Gum antioxidant capability could be ascribed to its amino acids, which have robust potential to quinch ROS and promote the oxidant scavenging system (Ali et al., 2020; Avelino et al., 2022). Additionally, it has anti-inflammatory and immunomodulatory properties owing to its potential to form short chains of fatty acids, which alter the inflammatory mediators release (Eslick et al., 2022). Accordingly, there is a growing body of literature has highlighted the effectiveness of Gum against renal diseases and various toxicants, including; chronic kidney disease (Ali et al., 2020), gentamicin (Ahmed et al., 2022; Ali et al., 2003), mercuric chloride (Gado and Aldahmash, 2013), and cisplatin (Al-Majed et al., 2003).

Consequently, this study was done to evaluate the mitigating modulatory impacts of Gum toward the nephrotoxicity induced by AFB1 exposure. Kidney function parameters, levels of expression of oxidative stress and inflammation-related genes, apoptosis signaling pathways, and renal histology were evaluated.

2. Materials and Methods

2.1. Phytochemical analysis of Acacia senegal extract

2.1.1. Preparation of Acacia senegal extract

The methanolic extract of the Gum was made by mixing 10 g of Gum with 100 mL 85% methanol and maintained at ambient temperature (30 ± 2 °C) for 24 h. Then, this extract was filtered and concentrated under vacuum at 40 °C by a rotatory evaporator to provide crude extract. Methanol was used to reconstitute the crude extract for further analysis.

Table 1
The phytochemical compounds identified in Gum extract by GC-MS analysis.

No	Chemical name	Bioactive Compounds	Retention time (min)	Peak area (%)	Molecular formula	Molecular weight
1	L-Gala-L-ido-octose	Octose (Monosaccharide)	11.74	0.36	C8H16O8	240
2	1,4-Benzenediol, 2-(1,1-dimethylethyl)– 5-(2-propenyl)-	Phenylpropanes	14.83	1.05	C13H18O2	206
3	Benzoic acid, 3,5-bis (1,1-dimethylethyl)– 4-hydroxy -, ethyl ester	Benzoic acid	15.16	0.43	C17H26O3	278
4	Trans-Z-à-Bisabolene epoxide	Sesquiterpenoids	15.41	0.25	C15H24O	220
5	10,13-Octadecadiynoic acid, methyl ester	Linoleic acids	17.73	0.8	C19H30O2	290
6	(1-Methylundecyl)benzene	Fatty acid	20.69	0.81	C18H30	358
7	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	Triacylglycerols	21.20	0.63	C19H34O6	358
8	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Benzoic acid (Carboxylic acid)	23.01	55.26	C16H22O4	278
9	Hexadecanoic acid, methyl ester	Palmitic acid	23.43	6.75	C17H34O2	270
10	Phthalic acid, butyl undecyl ester	Phthalic acid	24.09	0.52	C23H36O4	334
11	Hexadecanoic acid	Palmitic acid	24.83	3.66	C16H32O2	256
12	1,25-Dihydroxyvitamin D3, TMS derivative	Vitamin D_3	25.33	0.34	C30H52O3Si	488
13	Cyclopropane octanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester	Cyclopropane fatty acid	26.35	1.53	C22H38O2	334
14	Octadecanoic acid, methyl ester (Methyl stearate)	Stearic acid	26.96	4.17	C19H38O2	298
15	Hexadecanoic acid, 1,1-dimethylethyl ester	Palmitic acid	27.22	1.23	C20H40O2	312
16	9- Octadecenoic acid	Oleic acid (Omega-9)	28.18	19.81	C18H34O2	282
17	Oxirane undecanoic acid, 3-pentyl-, methyl ester, cis	Fatty acid	30.29	0.61	C19H36O3	312
18	2,2,3,3,4,4 hexadeutero octadecanal	Fatty aldehydes	30.55	0.54	C18H30D6O	274
19	Methyl-9,9,10,10-d4-octadecanoate	Stearic acid	33.42	0.61	C19H34D4O2	302
20	3′,8,8′-Trimethoxy-3-piperidyl-2,2′-b inaphthalene-1,1′,4,4′-tetrone	Alkaloid compound	33.70	0.31	C28H25NO7	487

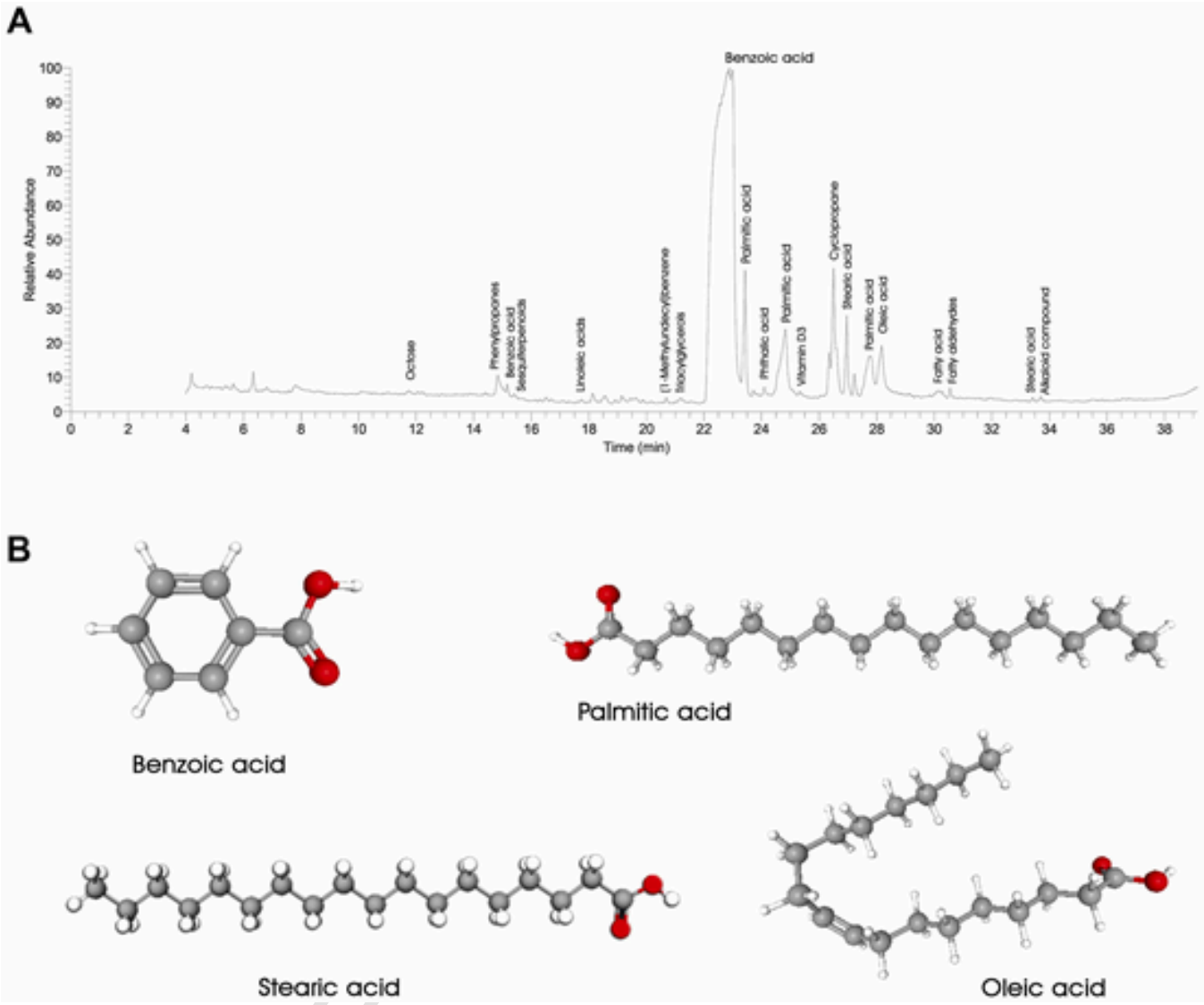

Fig. 1. GC/MS analysis of Gum extract. (A) GC/MS chromatogram expounds the abundance of distinct phytochemical constituents secluded at various retention times. (B) 3D Chemical structure of the main phytochemicals identified by GC/MS.

2.1.2. Gas chromatography-mass spectrometry (GC/MS) analysis of Acacia senegal extract

The phytoconstituents of Gum were identified by using GC/MS analysis (GC-Trace Ultra-ISQ mass spectrometer, Thermo Scientific, Austin, USA). The sample was injected to a capillary column (30 m × 0.2 mm × 0.25 μm) fused with silica. The injector temperature was maintained at 250 °C while the oven temperature of the GC was adjusted with an initial temperature of 60 °C/min and further increased at the rate of 10 °C/min until 280 °C with an overall holding time of 36.5 min. Helium (99.9% purity) was used as a carrier gas system with a regular flow rate of 1.0 mL/min. The solvent delay was 4 min and diluted specimens of 1 µl were injected automatically by autosampler (AS3000) coupled with GC and calibrated in the split mode. Within 40–650 *m/z* spectrum, electron ionization voltage was 70 eV at a full scan mode. The ion source temperature and GC–MS interface were maintained at 200 °C and 250 °C, respectively. The identity of active constituents was based on matching the retention times of identified compounds in the mass spectra with renowned compounds kept in the National Institute Standard and Technology (NIST) and WILEY mass spectral libraries. The obtained compounds have been tabulated. The percentage of composition was determined by area normalization.

2.2. Animal study and ethical endorsement

Wister albino rats (120 ± 10 g body weight) were procured from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Assiut University, Egypt. Rats were housed in appropriately adjusted environments (22.5 ± 2 °C, 50–60% humidity) for two weeks beforehand the experiment. All rats were fed on a conventional baseline diet with free access to water throughout the experiment. This experiment was granted by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, South Valley University (Approval no. 31B/14.04.2022).

After two weeks, rats were equally allocated to 4 groupings: (1) Control group, rats were received saline; (2) Gum group, in which rats was given gum by oral gavage at dose of 7.5 g/kg b.w once daily (Gado and Aldahmash, 2013); (3) AFB1 group, rats were exposed to AFB1 at a dose of 200 µg/kg b.w, orally, every alternate day (Tang et al., 2007); (4) AFB1-Gum, rats were co-treated with both gum and AFB1 as the doses mentioned above. Notably, AFB1 was given 5 h following gum administration.

After 4 weeks, the trial was terminated, the blood sample was gathered from the retro-orbital venous plexus, and the plasma was har-

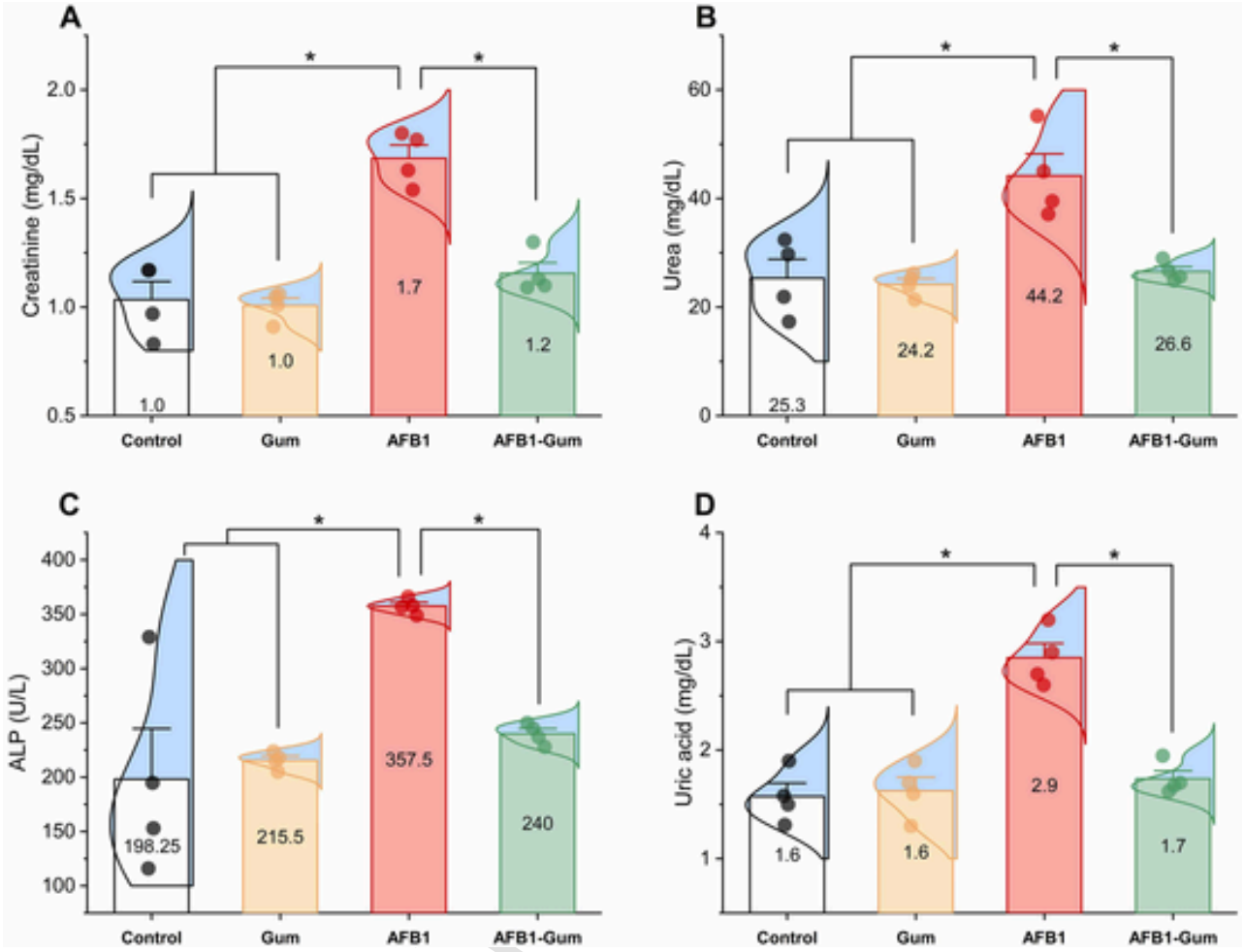

Fig. 2. Bar-dot plot panel of kidney function parameters for different groups treated with AFB1 and/or Gum. (A) Creatinine, (B) urea, (C) ALP, and (D) uric acid. All treatments were given orally with AFB1 were given 5 h after Gum administration. Bar plot is overlapped with the jitter data point and Kernel Smooth density plot. * denotes significant different, $p < 0.05$. Values were expressed as mean ± SE (n = 4).

vested and stored at − 20 °C for further biochemical testing. Following, all animals were euthanized under inhalation of isoflurane and the kidney was swiftly removed and rinsed out with cold physiological saline to remove any clots, thereafter, sliced into several pieces. For subsequent histological inspection, one part was fixed in 10% neutral buffered formalin. Other tissue portions were used for RNA and protein extraction and maintained at − 80 °C.

2.3. Estimation of kidney function parameters

Kidney function parameters, including urea, creatinine, uric acid, and alkaline phosphatase (ALP) were evaluated in the plasma. All procedures were carried out in accordance with the manufacturer's (Laboratory Biodiagnostics Co., Giza, Egypt) protocols.

2.4. RNA isolation with reverse transcription-PCR

According to the manufacturer's instructions, total RNA was isolated from kidney homogenate using QIAzol Lysis Reagent (QIAzolTM, QIAGEN®, USA). Using a spectrophotometer, the samples' total RNA concentration and quality were evaluated (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific, USA). The ratio of 260–280 nm absorbance was used to determine the RNA quality. The miScript II RT kit (QIAGEN®, USA) was used to reverse-transcribe the extracted total RNA into cDNA. The cDNA was created from 1 μg RNA utilizing a primer (oligo (dT) primers, PrimeScript™, TaKaRa Bio Inc, CA, USA). The PCR using the primers (interleukin-6 (IL-6), tumor necrosis factor α (TNFα), inducible nitric oxide synthase (iNOS), and nuclear factor kB p65 (NF-κB/P65)) given in Table S1 was carried out using a thermal cycler (A200 Gradient Thermal cycler, LongGene®, Hangzhou, China). The PCR products were then electrophoretically separated 1.5% agarose gel stained in ethidium bromide (Scientific Limited, Northampton, UK) in tris-borate-EDTA (TBE) buffer. The isolated bands were spotted by a gel recording system (Bio-Rad, USA) and band intensity was measured and normalized against β-actin by using the NIH Image J v1.47 program.

2.5. Western blotting assessment

Protein fraction was isolated from the organic phase fatty tissue samples processed with QIAzol Reagent-(QIAGEN®, QIAzol™) in accord to the manufacturer's instructions, and it was then treated with a proteinase inhibitor cocktail (Sigma-Aldrich, Germany) and phosphatase inhibitor tablet (PhosStop™, Roche Diagnostics, USA). Protein samples were loaded in equivalent proportions, isolated using SDS-poly acrylamide gel (SDS-PAGE) electrophoresis, and then blotted on a polyvinylidene difluoride membrane (PVDF, Immobilon-P, Millipore). The membranes were probed with the diluted primary antibodies (the nuclear factor erythroid 2–related factor 2 (Nrf2), IL-6, superoxide dismutase type 1 (SOD1), cytochrome c (Cyto c), and cleaved Caspase3 (Cl. Casp3–17/19)) after being blocked in PBS-Tween (0.1%) with 1% BSA (Table S2). The bands were detected using the BioRAD chemidoc

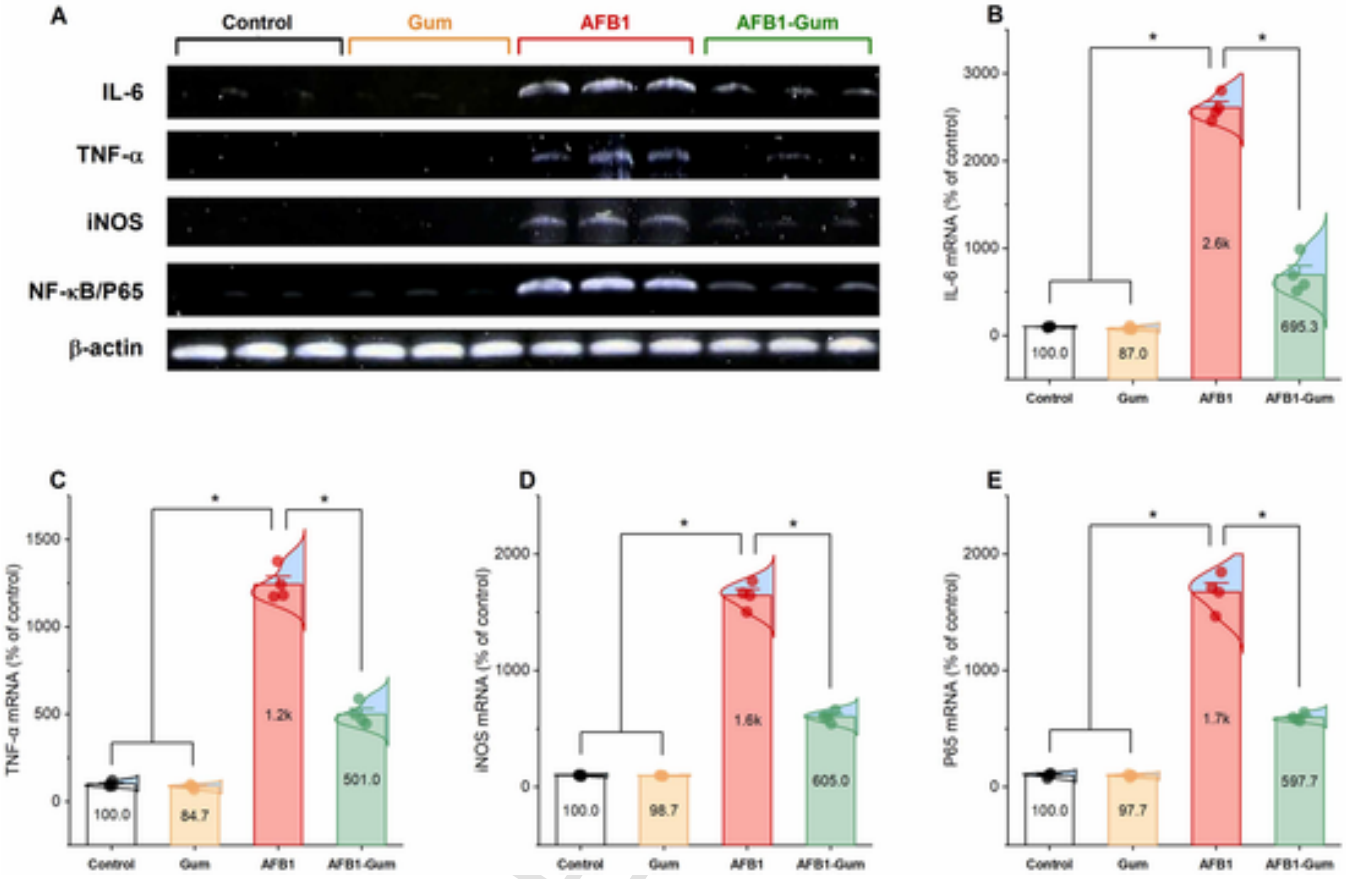

Fig. 3. Effect of AFB1 and/or Gum administration on renal mRNA expression of pro-inflammatory cytokines. (A) IL-6, TNF-α, iNOS, NF-κB/P65, and β-actin genes representative bands. (B) Bar-dot plot panel mRNA levels of proinflammatory cytokines by semi-quantitative analysis. β-actin served as an internal control. All treatments were provided orally and AFB1 was given 5 h after Gum administration. Bar plot is overlapped with the jitter data point and Kernel Smooth density plot. * denotes significant different, $p < 0.05$. Values were expressed as mean ± SE ($n = 4$).

and the Roche Lumi-light Plus kit. Bands intensities were quantified with the NIH Image J software.

2.6. Histological inspection and scoring

The formalin-fixed kidney tissue specimens were first dehydrated in ascending alcohol grades. Then xylene clearing was done before being embedded in paraffin. The tissue was sliced into 5 μm thick sections then stained with H&E for histoarchitecture inspection and scanned using a camera-integrated digital imaging system (DM300, Leica, Germany). According to Qi and Wu (Qi and Wu, 2013), the tubular injury was defined as tubular epithelial necrosis, lymphocytic infiltrations, cytoplasmic vacuolization, tubular dilatation and the loss of the brush border. Tubular injury was scored by grading the percentage of damaged renal tubules under ten randomly selected, non-overlapping fields at magnification of X200) as follows: 0, 0%; 1, ≤ 10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; and 5, 76–100%. To score injured tubules, whole tubule numbers per field were considered as standard at same scale. The grading percentage was calculated in each field as follows:

$$\text{Injuryscore}\,(\%) = \frac{\text{numberofinjuredtubules}}{\text{totalnumberoftubules}} \times 100$$

2.7. Data analyses

One-way analysis of variance (ANOVA) was used to analyze the data, and Duncan's post hoc test was used to compare the treatment means (SPSS software, version 21; Inc., Chicago, IL, United States). All values are regarded as statistically significant at $p \leq 0.05$ and expressed as the mean and 95% confidence interval. The OriginPro software (version 2019b) was used to for univariate data visualization. The MetaboAnalyst program was also used to create the dendrogram and a correlation heatmap.

3. Results

3.1. Chromatographic pattern of GC/MS spectral analysis of Acacia senegal extract

The detected compounds with their chemical formula, peak area, retention time, and molecular weight were displayed in Table 1 and Fig. 1. Overall, 20 peaks of the compounds were detected, including 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (55.26%), 9-octadecenoic acid (19.81%), hexadecenoic acid-methyl ester (6.75%), octadecanoic acid-methyl ester (4.17%), and hexadecanoic acid (3.66%), which were being the most prevailing. Those compounds that are retrieved from a methanolic extract of Gum are belonged into various chemical categories, mainly benzoic acid, oleic acid, palmitic acid, stearic acid, cyclopropane fatty acid, phenylpropanes, monosaccharides, linoleic acids, vitamin D_3, aldehydes, alkaloid, and sesquiterpenoids.

3.2. Renal function parameters

As illustrated in Fig. 2, AFB1-intoxication induced kidney injury, evidenced by a marked increase in renal function tests (urea, creatine, and uric acid level) (with mean = 44.2, 1.7 and 2.9, respectively) compared to control rats (with mean of urea = 25.3, creatinine = 1.0 and 1.6 uric

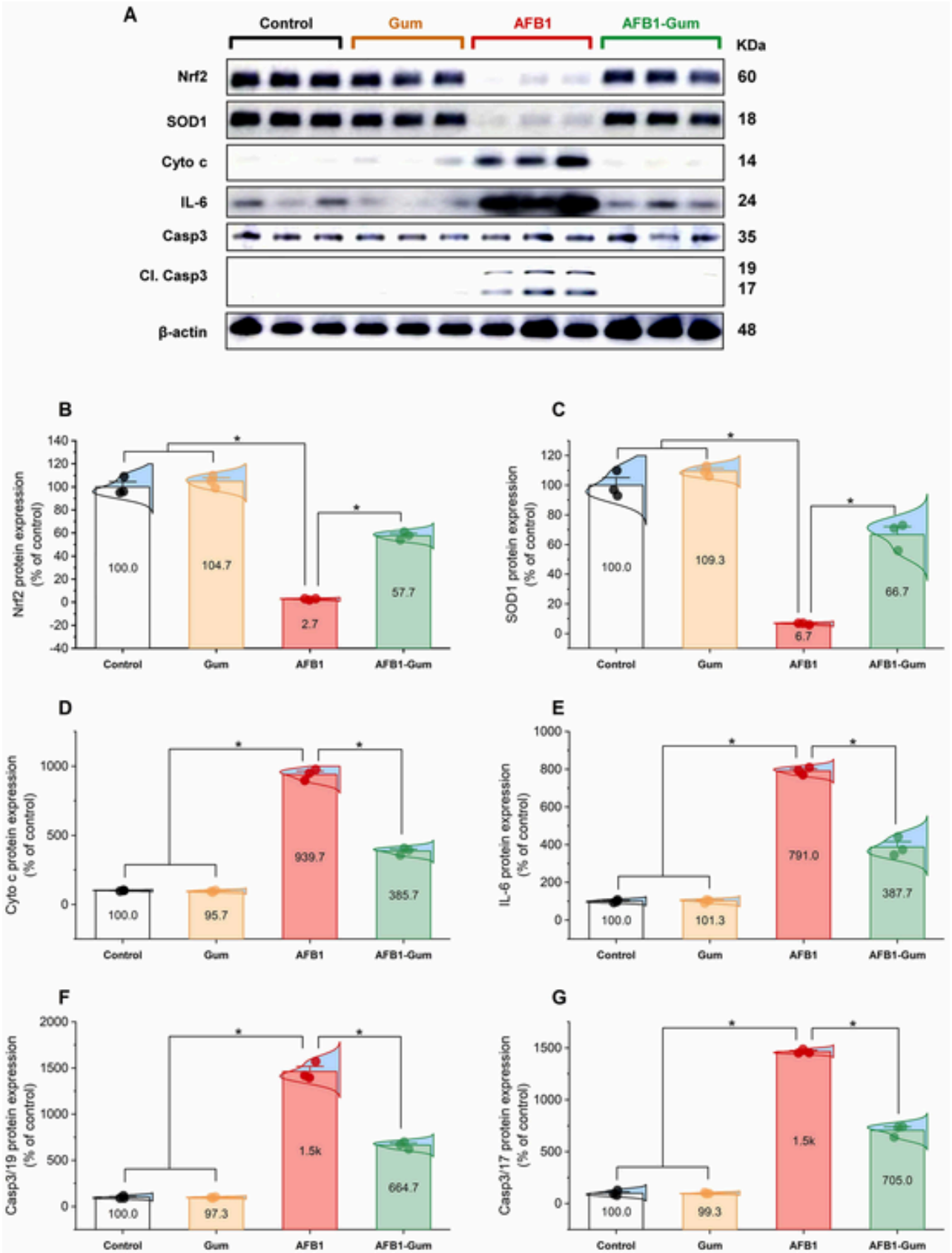

Fig. 4. Effect of AFB1 and/or Gum administration on protein expression levels of pro-inflammatory cytokine, antioxidant, and apoptotic markers in the kidney. (A) IL-6, Nrf2, SOD1, Cyto c, Cl. Casp3–17/19, Casp3, and β-actin proteins typical immunoblots. (B) Bar-dot plot panel were created from immunoblot by semi-

Fig. 4.—*continued*
quantitative analysis. β-actin functioned as internal control. All treatments were provided orally and AFB1 was given 5 h after Gum administration. Bar plot is overlapped with the jitter data point and Kernel Smooth density plot. * denotes significant different, $p < 0.05$. Values were expressed as mean ± SE (n = 3).

acid). Additionally, a remarkable increase in ALP enzyme activity (mean = 357.5) was spotted that implied AFB1-induced renal tissue damage. On the contrary, Gum supplementation could significantly lessen the renal tissue injuries brought on by AFB1 exposure (with mean of urea = 26.6, creatinine = 1.2, uric acid = 1.7 and ALP = 240).

3.3. Renal expression of proinflammatory cytokines

As seen in Figs. 3 and 4, exposure to AFB1 triggered an inflammatory response in the kidney tissue as evidenced by a marked upregulation of the levels of the mRNAs for TNF-α, IL-6, iNOS, and NF-kB/P65 (means = 1200, 2600, 1.6 and 1700, respectively) as well as an increase in protein expression of the IL-6 (mean = 791.0) in confront to controls. On the other hand, when AFB1-exposed animals were administered the Gum, mitigation of AFB1 toxic effect was observed, elucidated by amendment of the expression levels of proinflammatory cytokines' mRNA of TNF-α, IL-6, iNOS, and NF-kB/P65 (means = 501.0, 695.3, 605.0 and 597.7, respectively) and protein expression of the IL-6 (mean = 387.7).

3.4. Assessment of renal oxidative state

The protein expression levels of Nrf2 and SOD1 in renal tissue demonstrated a substantial downregulation, revealing oxidative stress initiation following exposure to AFB1 (with means = 2.7, and 6.7, respectively (Fig. 4). On the other hand, Gum supplementation significantly reduced AFB1-induced oxidative renal damage (mean of Nrf2 = 57.7 and SOD1 = 66.7).

3.5. Evaluation of apoptosis pathway in kidney

Fig. 4 shows apoptotic marker protein expression modifications in response to AFB1 and/or Gum administration. In comparison to control rats, AFB1 intoxication substantially increased the regulation of Cyto c protein and Cl. Casp3–17/19 (means = 939.7, 1.5k and 1.5k, respectively) indicating stimulation of the apoptotic cell death. On the other hand, Gum supplementation dramatically reduced the AFB1-induced apoptosis via regulating the expression levels of these proteins in renal tissue (means = 385.7, 705.0 and 664.7, respectively).

3.6. Hierarchical clustering dendrogram and correlation heatmap

Next, a hierarchical clustering dendrogram was applied to give a visual snapshot of our data. The current dendrogram (Fig. 5a) depicted that AFB1-exposed animals were descended from a separated branch discriminating them from other treated animals. However, the Control, Gum, and AFB1-Gum groups were clustered under the other branch of the dendrogram tree. These data suggested Gum supplementation to the AFB1-intoxicated animals could allocate this group of animals to the side of normal ones, unlike other rats that receive AFB1 only. These results explicate how the AFB1-intoxicated animals had more renal damage than the other animals.

Alongside, the correlation heatmap expounded in Fig. 5b, provides a 2D correlation matrix between all measured parameters, using colored cells to represent intuitive visualization of all the data sets. A correlation heatmap shows that the Nrf2 and SOD1 protein expression had a high positive correlation with each other, while a highly negative correlation between them and the expression of other apoptotic biomarkers (Cyto c and Cl. Casp3–17/19) and inflammatory markers (IL-6, TNF-α, iNOS, and NF-κB/P65). These findings suggested that oxidative stress, inflammation, and apoptosis are strongly associated with AFB1-induced renal injury.

3.7. Histopathological findings of kidney tissue inspection

For further affirmation of the previously presented data, a histopathological examination was carried out to assess the changes in renal histology following AFB1 and/ or Gum treatment. The control and Gum-treated animals showed normal architecture of renal tissue (renal corpuscle, proximal and distal convoluted tubules, and collecting ducts), as illustrated in Fig. 6. On the contrary, rats intoxicated by AFB1 showed overt renal damage, indicated by disintegration of brush border, cytoplasmic vacuolization of their lining epithelial cells, tubular dilation, and increased permeation of inflammatory cells was also observed. Nevertheless, synchronous administration of AFB1 and Gum resulted in an amendment of renal architecture near to normal, as evidenced by slight cytoplasmic vacuolation in some renal tubules with minimal loss of brush border and inflammatory cell leakage. The data obtained from the histological scoring revealed attenuation of AFB1-induced tissue damage in animals co-treated with Gum.

4. Discussion

AFB1 is of great global disquiet as it is considered the highest robust mycotoxin. AFB1 is predominantly consumed through diet since it frequently contaminates cereals and feedstuffs and remains persistent following food processing, thus posing serious health consequences (Wang et al., 2022). It has been implicated in various studies as a potential cause of renal disorders, where its metabolites are preferentially deposited in kidney tissue during urinary excretion (Wang et al., 2022; Yilmaz et al., 2018).

Tremendous evidence strongly implies that excessive free radical generation, oxidative stress, and activation of inflammatory cytokines are considered indispensable pathways in AFB1 renal toxicity (Abdel-Daim et al., 2021). Consequently, oxidative stress more highlighted in the current study, evidenced by the downregulation of Nfr2 and SOD1 protein expression. The Nrf2 signalling pathway is a crucial transcription factor for oxidative stress and redox status (Guo et al., 2022). It enhances the antioxidant potential of cells through the increased expression of protein levels of many intracellular enzymatic antioxidants, including SOD1 (Wang et al., 2022). The ongoing study also further affirmed the harmful effect of AFB1-induced oxidative stress on the tubular epithelial membrane. This indicated by the corruption of the brush border of cortical tubules, as shown in histological assessment. Consequently, a tubular dysfunction occurred evidenced by a substantial elevation in urea, creatinine, and uric acid serum levels. Importantly, mitochondria are the main target of oxidative damage. Therefore, the larger density of mitochondria in the proximal tubules enhanced their susceptibility to ROS-induced oxidative damage and apoptotic changes (Abdelnaby et al., 2022). These findings considerably corroborate our prior reports which showed that the cortical tubules are more susceptible to oxidative damage brought on by cefepime (Aboubakr et al., 2021), gentamicin (Abdeen et al., 2021), cadmium (Abdelnaby et al., 2022) and aflatoxin (Abdel-Daim et al., 2021). Taken together, our findings imply that AFB1 evidently induce renal insufficiency indicated by renal function deterioration and substantial histological degenerative modifications of the renal tissues. This findings were in concordance with beforehand investigations (Gao et al., 2021; Wang et al., 2022).

Inflammation and oxidative stress are strongly correlated, according to mounting evidence, hence, we propose that the inflammatory path-

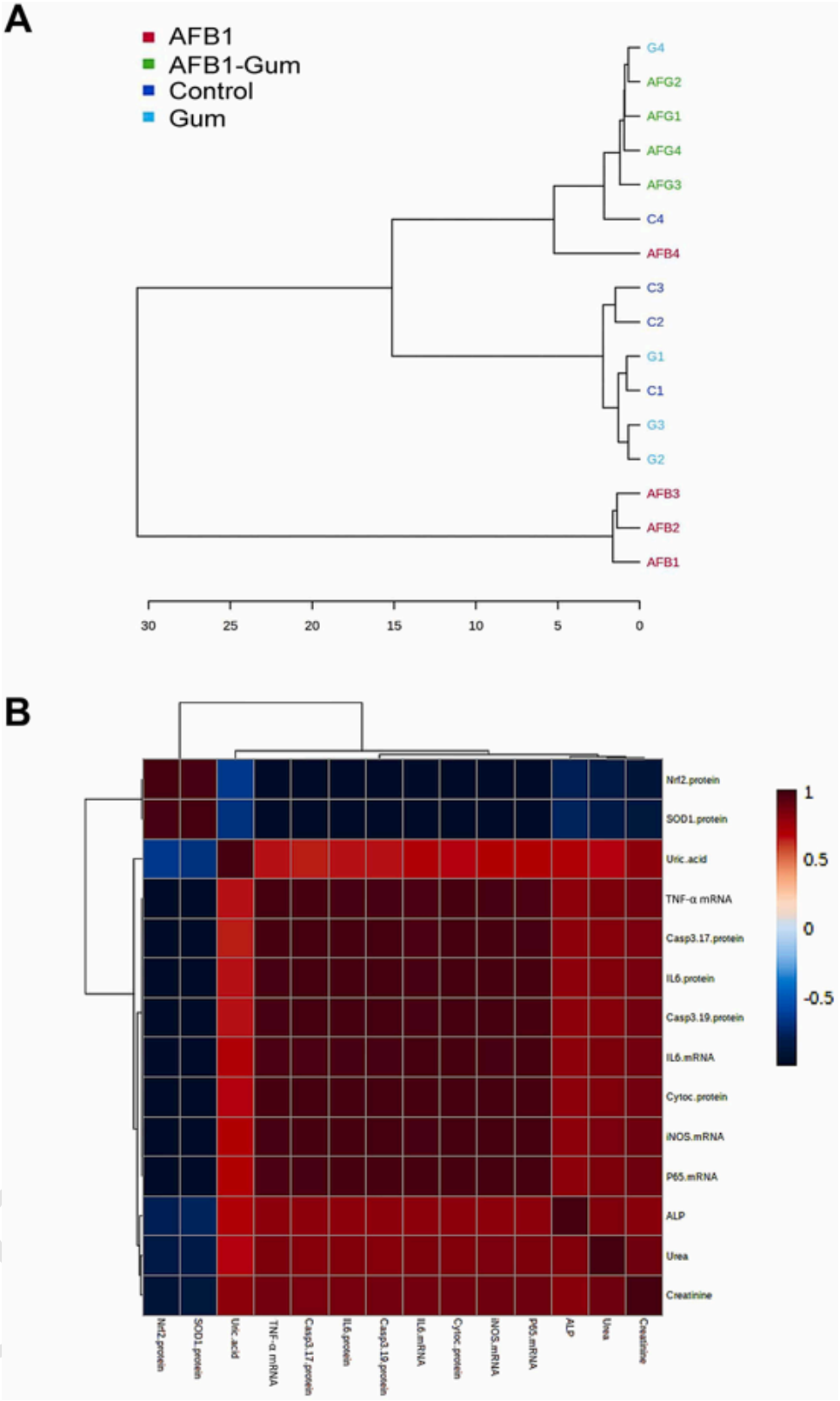

Fig. 5. Data clustering analysis of Gum against AFB1-prompted renal damage. (A) Dendrogram for cluster analyses employing average relationship between groups. The horizontal axis on the dendrogram plot gives the clustering distance, while the vertical axis displays the individual points that are collected into clusters (treated groups). Each node in the cluster tree holds a group of similar data. (B) Correlation heatmap between all measured variables. The contribution is explicated by a colored scale ranging from the highest (red indicates positive correlation) to lowest (blue indicates negative correlation). The degree of correlation is shown by the color's intensity. The various squares are colored in accordance with the Pearson's correlation coefficient between all measured variables.

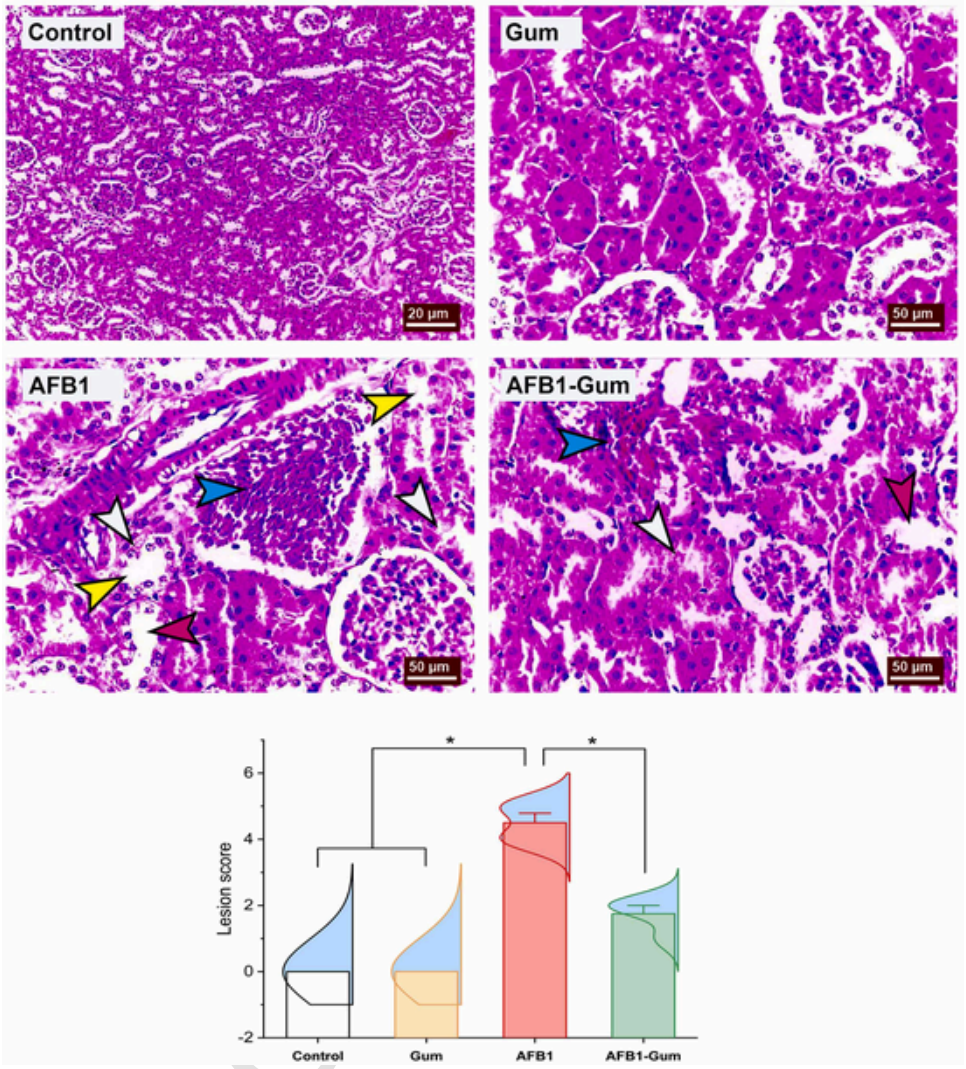

Fig. 6. Kidney tissue histology in Control, Gum, AFB1, and AFB1-Gum groups. The normal architecture of renal tissue was observed in the Control, and Gum-treated groups; AFB1, kidney section of AFB1-intoxicated group showed cytosolic vacuolation (white arrow) with degeneration of brush border of renal tubules (red arrow), tubular dilation (yellow arrow), and overt inflammatory cellular infiltration (blue arrow); AFB1-Gum rats displayed substantial amendment of renal parenchyma close to normal evidenced by slight vacuolation and loss of brush border with minimal inflammatory cell infiltration. The bar plot shows the lesion scoring of histopathological alterations of renal cells among different treated groups. Bar plot is overlapped with a Kernel Smooth density plot. * denotes significant different, $p < 0.05$. Values were expressed as mean ± SE.

way is a further mechanism contributing in AFB1-induced damage (Zhang et al., 2020). Enhanced generation of ROS is anticipated to commence an intracellular signaling pathway, upregulate the proinflammatory genes expression, and release the inflammatory mediators, culminating in an intense inflammatory response (Owumi et al., 2020). TNF-α and NF-κB/P65 are the most paramount inflammatory cytokine implicated in inflammation pathogenesis since. It activates proinflammatory mediators, and causes leukocytes to gather at the site of inflammation (Benkerroum, 2020; Cruceriu et al., 2020). Regrettably, iNOS boosts NO production along with the production of harmful peroxy-nitrite species (ONOO−), which enhances inflammation and necroptosis (Abdeen et al., 2021; Owumi et al., 2022; Lee et al., 2019). Histological examination of renal tissue vividly confirmed these effects, as evidenced by notable inflammatory cell spillage. Our correlation heatmap further confirms the strong association of oxidative stress and inflammation with AFB1 -induced renal injury. These data are congruent with previous investigations showing increased mRNA expression of these inflammation-associated genes (TNF-α, NF-κB/P65, IL-6, and iNOS) after AFB1 exposure (Gao et al., 2021).

AFB1 exposure is also associated with aberrant apoptosis since excessive ROS production triggers the mitochondrial-dependent apoptotic pathway. This happen through opening permeability transition pores in the inner mitochondrial membrane, leading to the breakage of the outer membrane (Gao et al., 2021; Wang et al., 2022; Meki et al., 2001; Kumar et al., 2022; Weiss et al., 2003). Subsequently, the apoptotic signaling molecules, Cyto c, egress from the intermembrane space into the cytoplasm, which triggers caspase cascades and apoptotic cell death (Meki et al., 2001; Owumi et al., 2022; Mughal et al., 2017). In addition, activation of NF-κB/P65 in the mitochondria is another mechanism that promotes the release of Cyto c that confirm the role of inflammation in instigating the apoptotic pathway (Albensi, 2019). According to the present investigation and preceding reports, AFB1-induced considerable upsurge of protein expression of apoptotic markers (Cyto c, Casp3–17, and Casp3–19) thus, strongly supporting the crucial role of apoptosis in AFB1-induced renal damage (Wang et al., 2022).

Gum has been shown to have a number of therapeutic potentials, comprising antioxidant, anticancer, immunomodulatory, cytoprotective and anti-inflammatory capabilities that are commonly utilized in Arabic conventional medicine (Ashour et al., 2022; Omer et al., 2021).

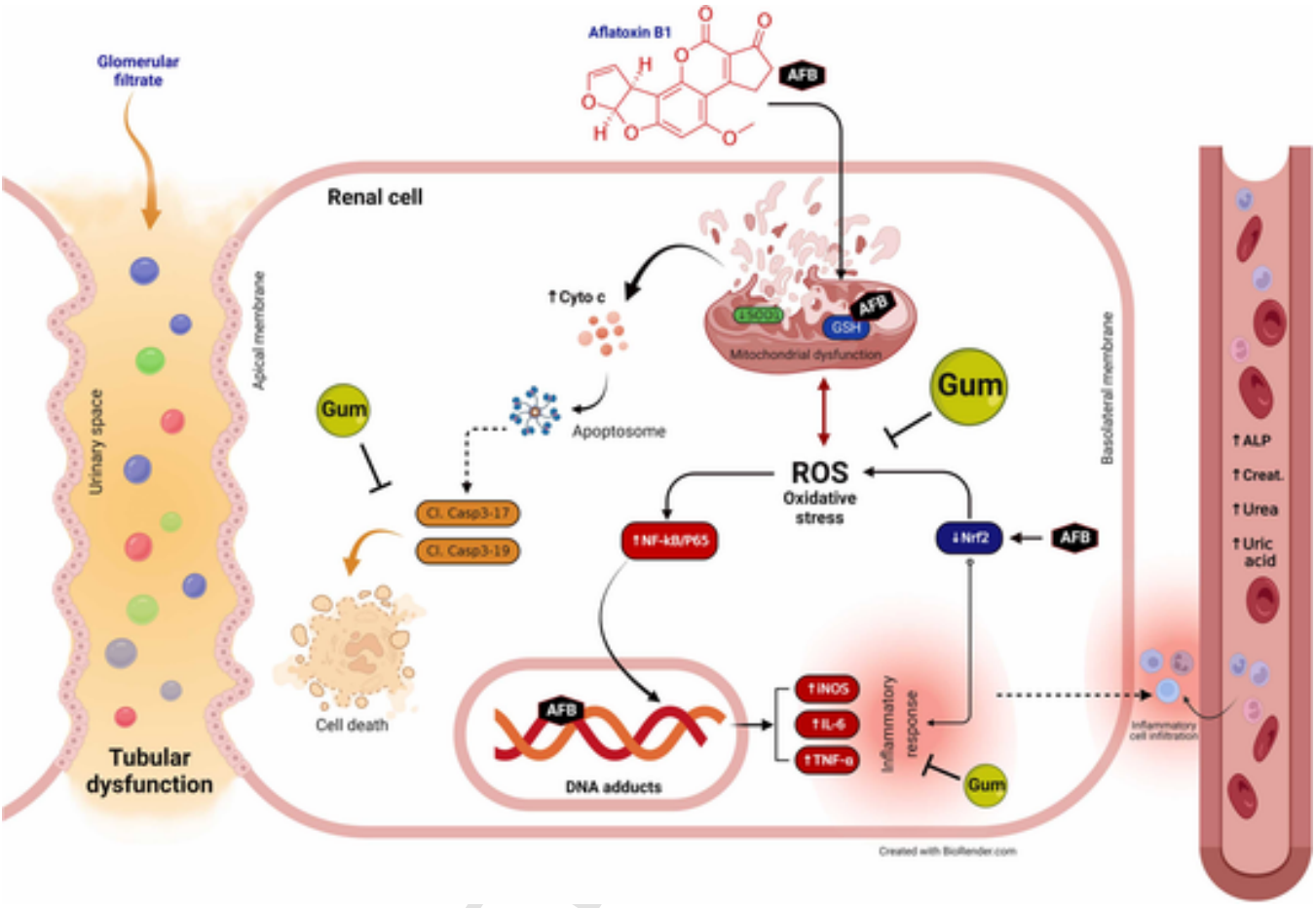

Fig. 7. Schematic design of the underpinning molecular pathway of the protective effect of Gum upon AFB1-induced renal damage.

The pharmacological properties of Gum could be attributed principally to the biological activities of its phytoconstituents (Ashour et al., 2022; Mirghani et al., 2018). Accordingly, GC/MS was used to examine the chemical constituents of the Gum. On the whole, the Gum was plenteous in phenolic compounds such as carboxylic acid, oleic acid, palmitic acid, and stearic acid. The antioxidant potential of these phenolic acids is based on the number and the position of hydroxyl groups on the benzene skeleton, which serve as electron-donating groups and are directly correlated with its free radical scavenging capability (Chen et al., 2020; Velika and Kron, 2012). In particular, benzoic acid is the most abundant compound and showed remarkable antioxidant and anti-inflammatory activities (Kumar et al., 2022; State and Unit, 2018). Its antioxidant ability is owing significantly to the presence of carboxylic acid groups (Chen et al., 2020) that can directly reduce ROS, boost antioxidant enzyme activity by influencing signal transduction pathways and regulate the expression of antioxidant genes (e.g., redox-sensitive Nrf2 system) (Godlewska-żyłkiewicz et al., 2020). On the other hand, it has a potent inhibitory effect on COX-2 and histone deacetylases (HDACs) enzymes, which are crucial in modulating the expression of inflammatory genes, thus, possess a potential anti-inflammatory effect (Godlewska-żyłkiewicz et al., 2020).

Noteworthy, the ongoing study explicates that pre-remediation with Gum could potentially mitigate the renal damage induced by AFB1, as evidenced by dramatical betterment in renal function tests. Gum has a salutary effect on renal function by utilizing the bowel as a "kidney substitute" (Ali et al., 2013). It can simulate colonic bacteria to generate ureases that catalyze the hydrolysis of urea into NH_3 and CO_2, boosting its excretion in stools (Ashour et al., 2022). This is congruent with preceding reports, that confirm the reduction of serum urea and creatinine level in case of chronic renal failure (Ali et al., 2020) and diabetic nephropathy (Ashour et al., 2022). The current work has also noticed an immunomodulatory effect of Gum in the AFB1-intoxicated rats, which was expounded by refinement of the expression level of proinflammatory cytokines and obscuring inflammatory cell infiltrations in renal tissues. Intriguingly, fermentation of Gum by intestinal microbiota produces abundant quantities of short chain fatty acids, notably, which significantly inhibit the release of proinflammatory cytokines (Ali et al., 2020; Omer et al., 2021). Our findings are similar to other antecedent reports (Hassanien, 2019; Elshama, 2018). In addition, our study revealed that Gum remedy could provide a sturdy antioxidant effect versus AFB1-induced oxidative stress. The protective aptitude of Gum to scavenge free radicals is ascribed to the abundance of its bioactive phenolic ingredients, as expounded above in GC/MS study. The Gum's antioxidant activity is one of the most plausible mechanisms contributing to its beneficial action against kidney damage (Gado and Aldahmash, 2013). Besides the antioxidant capacity, Gum supplementation also divulged an anti-apoptotic impact against AFB1-prompted increased caspase-3 expression as shown in our and preceding study (Gao et al., 2021).

We used a hierarchical clustering dendrogram to summarize the diverse contribution of various treatments to renal tissue. AFB1 exposed group was significantly different from all other groups. These results explicate how the AFB1-intoxicated animals had more renal damage than the other animals. The molecular mechanisms for the mitigating action of Gum after AFB1 intoxication are depicted in Fig. 7.

5. Conclusions

AFB1 provoked severed renal damage via triggering the oxidative cascade, inflammatory reaction, and apoptotic pathway. *A. senegal* supplementation could ~~potentially~~ provide a nephroprotective action against AFB1 -inflicted renal damage. A. senegal maintained the integrity of renal tissue architecture and its function, alleviated AFB1-induced oxidative stress, inflammatory reaction, and apoptotic death, inhibited the NF-κB pathway, and boosted the Nrf2 signaling pathway. Therefore, we anticipate that *A. senegal* can be a dietary supplement of compatible therapeutic importance for alleviating AFB1-induced kidney injury.

Institutional Review Board Statement

The animal study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, South Valley University approved the use of experimental animals and the study design (Approval No. 31b/14.04.2022).

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CRediT authorship contribution statement

Obeid Shanab : Writing – review & editing, Visualization, Supervision, Project administration, Conceptualization, Methodology, Investigation, Formal analysis, Resources, Writing– original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Samir M. El-Rayes :** Conceptualization, Methodology, Validation, Writing – original draft. **Waleed F. Khalil :** Conceptualization, Validation, Writing – original draft. **Noha Ahmed :** Conceptualization, Methodology, Investigation, Writing – original draft. **Afaf Abdelkader :** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Nashwa H. Aborayah :** Methodology, Formal analysis, Data curation, Writing – original draft. **Ahmed M. Atwa :** Validation, Data curation, Writing – original draft, Visualization. **Faten I. Mohammed :** Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Hend E. Nasr :** Methodology, Investigation, Data curation, Writing – original draft, Visualization. **Samah F. Ibrahim :** Software, Resources, Writing – original draft, Visualization. **Amr M. Khattab :** Software, Resources, Writing – original draft, Visualization. **Mohammed Alsieni :** Software, Validation, Resources, Writing – original draft. **Ali Behairy :** Investigation, Formal analysis, Data curation, Writing – original draft. **Liana Fericean :** Software, Formal analysis, Resources, Writing – original draft, Writing – review & editing. **Lina A. Mohammed :** Methodology, Investigation, Formal analysis, Writing – original draft, Visualization. **Ahmed Abdeen :** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Samah F. Ibrahim reports financial support was provided by Princess Nourah bint Abdulrahman University. Liana Fericean reports financial support was provided by University of Life Sciences.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115194.

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