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RESEARCH

An *in vitro* experimental study on the interference of glyphosate on the urease enzyme

Glifosatın üreaz enzimi üzerindeki interferansına ilişkin *in vitro* deneysel bir çalışma

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Abstract

Purpose: Exposure to glyphosate is increasing due to the density of agricultural areas in Türkiye. In this study, the possible interference effect of glyphosate on urease, an enzyme that is frequently used in the diagnosis and follow-up of many diseases and in the measurement of urea in biological samples was examined.

Materials and Methods: First, glyphosate was observed to have a negative interference in experiments using solutions of varying concentrations of urea. Second, blood samples were examined using the urease-glutamate dehydrogenase (GLDH) and indirect nesslerization procedures to determine the effects of glyphosate on the results before and after its addition. To determine the morphological and chemical alterations, scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR) analyses were conducted, and binding patterns were established through molecular docking. Urea measurements conducted with urease-GLDH and indirect nesslerization demonstrated a negative interference on the results with glyphosate concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M.

Results: Morphological changes observed in the SEM analysis were supported by the 3228.25 (O-H), 1642.08 (C=C), and 1531.20 (N-O) cm^{-1} bonds formed in the FTIR analysis. Furthermore, the molecular docking analysis showed that glyphosate affected the urease via hydrogen bonding (Gly13, Ser12, Lys14, Thr15, and Asp37) and hydrophobic interactions (Val10, Asp37, and Glu98). It was hypothesized that these interacting amino acids limit the accessibility of the urease's active catalytic conformation and/or impact the stability of the catalytic transition state.

Conclusion: Glyphosate leads to negative interference in

Öz

Amaç: Türkiye'de tarım alanlarının yoğunluğu nedeniyle glifosata maruziyet artmaktadır. Bu çalışmada glifosatın birçok hastalığın tanı ve takibinde üre ölçümünde sıklıkla kullanılan bir enzim olan üreaz üzerindeki olası interferans etkisi incelendi.

Gereç ve Yöntem: Ön denemelerde glifosatın, değişen konsantrasyonlarda üre solüsyonlarıyla negatif etkileşime sahip olduğu gözlendi. İn vitro ortamda glifosat eklenmesi öncesi ve sonrası sonuçlara etkisini belirlemek amacıyla kan örnekleri üreaz-GLDH ve dolaylı nesslerizasyon prosedürleri kullanılarak incelendi. Morfolojik ve kimyasal değişiklikler için taramalı elektron mikroskobu (SEM) ve Fouirer-Transform Infrared Spektrofotometre (FTIR) analizleri yapıldı ve moleküler kenetleme yoluyla bağlanma modelleri oluşturuldu. Üreaz-GLDH ve dolaylı nesslerizasyon ile gerçekleştirilen üre ölçümleri, 10^{-3}, 10^{-4}, ve 10^{-5} M konsantrasyonlarında glifosattaki sonuçlar üzerinde negatif bir etkileşim göstermiştir.

Bulgular: SEM analizinde gözlenen morfolojik değişiklikler, FTIR analizinde oluşan 3228.25 (O-H), 1642.08 (C=C), ve 1531.20 (N-O) cm^{-1} bağları ile desteklenmiştir. Ayrıca moleküler kenetleme analizi, glifosatın, üreazı hidrojen bağı (Gly13, Ser12, Lys14, Thr15, ve Asp37) ve hidrofobik etkileşimler (Val10, Asp37, ve Glu98) yoluyla etkilediğini göstermektedir. Bu etkileşimli amino asitlerin, üreazın aktif katalitik konformasyonunun erişilebilirliğini sınırladığını ve/veya katalitik geçiş durumunun stabilitesini etkilediği öngörülmüştür.

Sonuç: Glifosat, insan serum üre testlerinde negatif etkileşime yol açarak klinik biyokimya, mikrobiyoloji ve tarım laboratuvarlarında hatalı test sonuçlarına yol açar. Analiz yapılırken bu etki dikkate alınmalı ve klinisyenlerin

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human serum urea assays, leading to incorrect test results in clinical biochemistry, microbiology, and agricultural laboratories. This effect should be considered when conducting analysis, and clinicians as well as hospital information management systems should be informed ahead of time, with special emphasis devoted to this interference.
Keywords:. Glyphosate, interference, urease

yanı sıra hastane bilgi yönetim sistemleri de bu müdahaleye özel önem verilerek önceden bilgilendirilmelidir.

Anahtar kelimeler: Glifosat, girişim, üreaz

INTRODUCTION

Urea is the most abundant non-protein nitrogenous molecule in the blood and is the principal metabolic result of nitrogen catabolism. The molecule is made up of free ammonia, which is formed from the amino groups of nitrogenous molecules in the urea cycle in the liver and amino acids in protein catabolism. The bloodstream transports the produced urea to the kidneys, where it is filtered from the plasma by the glomeruli. Urea is the main metabolic product of nitrogen catabolism and has the highest concentration of non-protein nitrogenous compounds in the blood. The molecule is comprised of free ammonia produced from amino groups of nitrogenous molecules in the urea cycle in the liver and amino acids in protein catabolism. The bloodstream transports the produced urea to the kidneys, where it is filtered from the plasma by the glomeruli [1]. The majority of the urea in the glomerular filtrate is excreted in the urine, while the remainder is reabsorbed during the transit of the filtrate through the renal tubules by passive diffusion. Less than 10% of the total urea is eliminated by the digestive tract and skin[2].

In clinical applications, abnormal plasma/serum urea concentrations may be classified as prerenal (congestive heart failure, shock, gastrointestinal hemorrhage, increased protein catabolism, high protein diet), renal (acute-chronic renal failure, glomerular nephritis), or postrenal (obstruction of the urinary tract). Consequently, urea measurement in clinical biochemistry laboratories is employed for the evaluation of renal functions, the determination of hydration status and nitrogen balance, the diagnosis of kidney-related disorders, and the confirmation of adequate dialysis[3].

Urease (urea amidohydrolase, EC 3.5.1.5) is a nickel-dependent metalloenzyme that is synthesized by plants, fungi, and bacteria but not by animals/humans. Pathogenic bacteria in the human gastrointestinal tract possess ureolytic characteristics, which catalyze the breakdown of urea into ammonia and is dependent on the urease enzyme. These bacteria may contribute to the pathophysiology of numerous disorders[4] (Table 1).

Table 1. Bacterial species with urease activity in humans, the diseases they cause, and their role in these diseases

Role of urease	Bacteria type	Disease	Reference
Persistence in the host cells	*Helicobacter pylori*	Gastritis, peptic ulcer, stomach cancer, anemia, insulin resistance	5, 6, 7, 8, 9
Lives in the host cells	*Mycobacterium tuberculosis*	Tuberculosis	10
	Escherichia coli	Hemolytic uremic syndrome, Crohn's disease	11, 12
Damage to the glycosaminoglycan layer	*Proteus mirabilis*	Urinary tract infection	13, 14, 15
	Staphylococcus saprophyticus, Staphylococcus aureus		
Survival in strongly acidic environments, high adhesion to intestinal cells	*Klebsiella pneumoniae*	Nosocomial infections including pneumonia and soft tissue infection	16

By stimulating the breakdown of urea into inorganic ammonia and carbon dioxide, urease plays a crucial role in the nitrogen cycle. X-ray crystallography have demonstrated that plant and bacterial ureases have a common 'trimeric' structure (Figure 1). The enzyme is a heteropolymer made up of three subunits named α (UreC), β (UreB), and γ (UreA), with the active centers situated in the α subunit [17]. The active center is coordinated by two nickel ions, a carbamylated lysine, four histidines, an aspartate residue, and three water molecules (Figure 1).

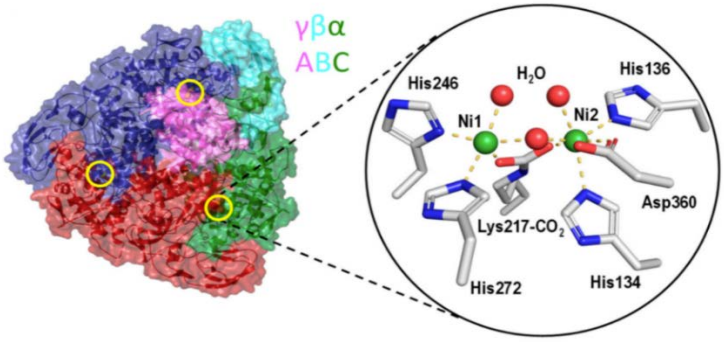

Figure 1. 3D structure of the urease enzyme [17]

$$H_2N\overset{O}{\overset{\|}{C}}NH_2\ (Urea) + H_2O \xrightarrow{Urease} 2NH_3 + CO_2 \quad (1)$$

In plants, all subunits are assembled into a polypeptide chain as UreA. The helix-turn-helix pattern in the subunit, known as the flap, is essential for the function of urease. According to the conformation of this motif, if the urea molecule is in the open position, it can reach the active site and hydrolysis can occur. In the closed position, the helix-turn-helix pattern prevents the urea molecule from entering the active site [18]. Eq. (1) depicts the general reaction mechanism of urea hydrolysis as catalyzed by urease.

Glyphosate (chemical name N-phosphonomethyl glycine ($C_3H_8NO_5P$), brand name Roundup) is the most extensively used broad-spectrum organophosphate herbicide in the world (Figure 2). Glyphosate is composed of phosphonic acid, which results from the formal oxidative coupling of the methyl group of methylphosphonic acid with the amino group of glycine [19]. Despite being synthesized for the first time in 1950, its herbicidal properties were only discovered in 1970. Since the 1980s, glyphosate has been increasingly employed to suppress cover crops and weeds. Globally, around 800,000 tonnes of glyphosate was utilized in agriculture per year as of 2014 [20]. The global consumption of glyphosate is anticipated to increase, possibly reaching 1 million tons by 2023. In the European Union, glyphosate was authorized for use until 2022, and procedures for re-evaluation are currently underway. While 300 tons of glyphosate were used in Türkiye in 2001, it has been projected that 8000 tons were used in 2019 [21].

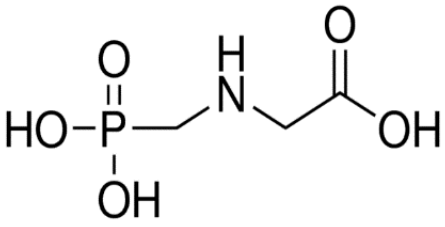

Figure 2. Chemical structure of glyphosate

Glyphosate inhibits the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is essential for the shikimate pathway. This enzyme is crucial for the synthesis of aromatic amino acids; hence, its inhibition affects protein production and causes plant death [22]. It has been reported that glyphosate exhibits a considerable chelating effect, particularly by readily binding divalent cations (such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, and Ni^{2+}) and creating stable complexes [23]. The use of glyphosate as a harvest aid results in high levels of glyphosate residue in crops, whereas pre-crop or post-harvest treatment of glyphosate results in detectable levels of glyphosate residue in crops less frequently [23,24].

Due to its extensive use, glyphosate residues and its principal metabolic product, aminomethylphosphonic acid (AMPA), have been detected in plants, soil, surface water, and groundwater. Humans are exposed to glyphosate through the consumption of plant-based foods, the meat of farm animals fed glyphosate-infected feed, and contaminated drinking water [25]. In recent years, glyphosate and its metabolites have been implicated in the development of numerous pathological conditions, including cancer, neurodegenerative diseases such as Autism, Parkinson's disease, and Alzheimer's disease, autoimmune diseases such as Crohn's disease and ulcerative colitis, obesity, and infertility [26].

A limited number of studies have been published on the effects of glyphosate has on the activity of enzymes in metabolic pathways. However, herbicides, pesticides, and insecticides used in agriculture can impact not just the target region, but also those who work there, live nearby, and consume the products. Agriculture is an important sector in Türkiye due to its contribution to employment, exports, and gross domestic product. Because of the abundance of agricultural areas in Türkiye, glyphosate exposure is growing. As a result of exposure and incorrect interpretation of results in laboratory settings, many xenobiotics can lead to diseases in humans. Interference is described as chemicals that modify the concentration or activity of an analyte in a sample tested in clinical biochemistry laboratories. The purpose of this study was to examine the potential interfering effect of glyphosate on the urease enzyme, which is used to evaluate the diagnosis and follow-up of numerous disorders and is commonly measured for urea in biological samples.

MATERIALS AND METHODS

Preliminary studies

First, the urease-glutamate dehydrogenase (GLDH) method was used to generate the standard curve for solutions with urea concentrations of 5, 10, 20, 40, 80, and 120 mg/dL. Then, 10^{-3}, 10^{-4}, and 10^{-5} M of glyphosate were added to the media, and the changes resulting from the urea concentrations were analyzed. These concentrations were selected based on toxic effect doses reported in the literature [27]. As a result of these analyses, negative interference of up to 60% was observed in repeated measurements of glyphosate with various urea concentrations, and serum-based studies were initiated.

Data collection and experimental procedure

Institutional review board approval (October 2022, meeting/decision no. 121/17) was precured before the study began from the Çukurova University Faculty of Medicine Non-invasive Clinical Research Ethics Committee. Urea was measured from random serum samples that were collected from laboratory samples whose routine analysis has been competed (n = 30). Analyses were carried out using two distinct urease enzyme techniques. In the first technique, the Randox UR220 kit and the urease-GLDH bienzymatic-linked system were used at 340 nm. The spectrophotometric approach measuring at 505 nm using the indirect nesslerization reaction was employed in the second technique. Different amounts of glyphosate were applied concurrently with the reaction reagents in both procedures.

Analysis via the urease-GLDH method

In the enzymatic kinetic method, urease hydrolyzes urea in the presence of water to form ammonia and carbon dioxide. In the presence of glutamate dehydrogenase, this produced ammonia reacts with 2-oxoglutarate, nicotinamide adenine dinucleotide (NAD), and reduced NAD (NADH) to produce glutamate and oxidized NAD (NAD^+). The formed NAD^+ concentration is proportional to the urea concentration in the environment and is evaluated as decreasing absorbance [28]. Measurements were performed by taking the average (ΔG) of the 1st, 2nd, and 3rd minutes at 340 nm on a Shimadzu UV 260 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The urease-GLDH reaction mechanism is given in Eq. (2):

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$
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$$2\,\alpha - Oxoglutarate + 2NH_4^+ + 2NADH_2 \xrightarrow{GLDH} L - Glutamate + 2NAD^+ + 2H_2O \quad (2)$$

In a repeatability analysis with n = 10 measurements in a serum sample containing 50 mg/dL of urea, the coefficient of variation value was 3.5%.

Analysis via indirect nesslerization

In the indirect nesslerization technique, the urease enzyme converts the urea in the serum to ammonium carbonate. The ammonium ion forms a yellow complex with Nessler's reagent and the color intensity formed at 505 nm is directly proportional to the measured ammonium-urea concentration in the medium [29].

Scanning electron microscope (SEM) analysis

For the SEM analysis, four groups of samples were prepared: urease, urease+urea, urease+glyphosate, and urease+urea+glyphosate. The initial concentrations of urease were 15 U/mL, urea was 50 mg/dL, and glyphosate was 10–1 M, with all volumes at 1:1. Then, 50 μL of each sample was applied to each microscope slide, dispersed evenly, dried in an incubator at 37 °C for 9 h, transferred into Eppendorf tubes, and prepared for examination. The analyses were carried out in the Central Research Laboratory of Çukurova University using an FEI Quanta 650 Field Emission type SEM microscope (FEI Company, Hillsboro, OR, USA).

Fourier-transform infrared spectroscopy (FTIR) spectrum analysis

Four groups of samples were prepared for the FTIR analysis: urease, urease+urea, urease+glyphosate, and urease+urea+glyphosate. FTIR was performed on some of the powder samples produced for the SEM examination. The analyses were carried out at the Central Research Laboratory of Çukurova University using a JASCO FT/IR-6700 spectroscope (JASCO Corp., Ishikawa-machi, Hachioji, Tokyo).

Computational molecular docking analysis

AutoDock 4.0 was used to perform molecular docking analyses to determine probable binding sites on the urease enzyme crystal structure of glyphosate (PDB code: 4HI0) [30]. The crystal structure of a 2.35 Å resolution urease enzyme was chosen as the target molecule. Before beginning the docking investigation, parameters were prepared using AutoDockTools (ADTs), urease, and glyphosate molecules. Polar hydrogen atoms were preserved in the urease enzyme and glyphosate molecules, while non-polar hydrogens were merged. Gasteiger loads with ADTs were used in the calculations [31,32]. During the molecular docking experiment, all rotatable bonds of the ligand were allowed to rotate, and then the prepared receptor and ligand structures were recorded in PDBQT format. A 60 × 60 × 60 grid box with a 0.375 grid spacing was established. Dockings were constructed utilizing 25 genetic algorithm runs, 5×10^{-5} energy evaluation counts, and a maximum of 27,000 generation iterations, with a starting population of up to 150 individuals. The population was assigned mutation and transmission rates with the values 0.02 and 0.80, respectively. After 100 individual docking trials for the glyphosate molecule, the algorithm clustered all conceivable binding modes. For the selected posture of the glyphosate, the conformation with the lowest binding free energy and the best clamping position was rated based on the binding free energy kcal/mol. Using BIOVIA Discovery Studio Visualizer 2016 (Dassault Systèmes, San Diego, CA, USA), the optimal docking pose between the ligand and receptor was assessed with AutoDock 4.0 [33].

Statistical analysis

All analyses were performed using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages, whereas continuous

variables were expressed as the mean ± standard deviation (SD) and the median and minimum–maximum where appropriate. The normality of distribution for the continuous variables was confirmed using the Shapiro–Wilk test. For comparison of the continuous variables between two groups, the Mann–Whitney U test was used. To evaluate the correlations, the Spearman's correlation coefficients were calculated. For non-normal distributed data, the Friedman test was used to compare the baseline and samples treated with 10^{-3} M, 10^{-4} M, and 10^{-5} M of glyphosate. The Bonferroni-adjusted Wilcoxon test was used for pairwise comparisons of the baseline and samples treated with 10^{-3} M, 10^{-4} M, and 10^{-5} M of glyphosate. Agreements between the results of the baseline and samples treated with 10^{-5} M of glyphosate were assessed using the Bland–Altman test with 95% confidence interval (CI) limits of agreement. R package ggplot2 was used to create the Bland–Altman plots. Statistical significance for all the tests was accepted as $p < 0.05$.

RESULTS

Table 2 depicts the urea distributions according to the concentrations. The patients' mean urea level was calculated as 19.5 ± 7.3 and 15.9 ± 4.7 when using the urease-GLDH and nesslerization, respectively ($p = 0.559$). In terms of the urea, a statistically significant difference was discovered between the different concentration groups with both the urease-GLDH and nesslerization ($p < 0.001$) (Figures 3 and 4). Post-hoc analyses revealed that, while all the pairwise comparisons with the nesslerization were statistically significantly different, only the baseline versus 10^{-5} M of glyphosate comparison with the urease-GLDH was not. When the correlation coefficients between the baseline and 10^{-3} M of glyphosate findings in all the patients were examined, it was discovered that there was a moderate association with the urease-GLDH ($r = 0.775$, $p < 0.001$) and nesslerization ($r = 0.761$, $p < 0.001$).

Table 2. Mean, median, and SD values and post-hoc comparisons of the urease-GLDH and nesslerization methods

Urease-GLDH	**Mean ± SD**	**p-value**
	median (min–max)	
Baseline	19.5 ± 7.3	<0.001
	12.3 (5.6–123.0)	
10^{-3} M	6.8 ± 2.0	
	4.9 (1.6–32.1)	
10^{-4} M	14.7 ± 6.8	
	7.9 (3.9–90.0)	
10^{-5} M	16.6 ± 6.9	
	10.0 (4.0–99.1)	
Nesslerization		**p-value**
Baseline	15.9 ± 4.7	<0.001
	11.3 (6.3–97.6)	
10^{-3} M	8.0 ± 2.8	
	5.3 (0.0–59.2)	
10^{-4} M	12.2 ± 3.5	
	8.7 (0.0–83.3)	
10^{-5} M	14.1 ± 4.5	
	9.7 (5.4–88.4)	

Data are expressed as the mean ± SD, median (min–max). In the post-hoc comparisons: for nesslerization, baseline versus 10–4 M, baseline versus 10–3 M, and 10–3 M versus 10–5 M: $p < 0.001$; baseline versus 10–5 M: $p = 0.023$, 10–4 M versus 10–5 M: $p = 0.043$, and 10–3 M versus 10–5: $p = 0.023$. For urease-GLDH, 10–3 M versus 10–5 M, baseline versus 10–4 M, and baseline versus 10–3 M: $p < 0.001$; 10–3 M versus 10–4 M: $p = 0.010$, 10–4 M versus 10–5 M: $p = 0.014$, and baseline versus 10–5 M: $p = 0.086$.

Figures 3 and 4 depict the box plot used to assess the correlation between the baseline and samples treated with 10^{-3} M, 10^{-4} M, and 10^{-5} M of glyphosate for all the patients. Because the differences were not normally distributed, the original data were transformed logarithmically. The mean differences obtained were 12.6 ± 7.6 and 7.9 ± 1.9 for urease-GLDH and nesslerization, respectively. In contrast to

the good agreement with 10^{-4} and 10^{-3} M of glyphosate, there was poor agreement between baseline and 10^{-5} M of glyphosate. The graphs of the Bland–Altman analysis performed to evaluate the agreement between baseline (no glyphosate) and 10^{-3} M of glyphosate (high concentration for this study) results for all the patients are shown in Figure 5. Since the differences were not normally distributed, logarithmic transformation of the original data was considered.Since the Spearman's correlation coefficients in Table 3 are 0.775 and 0.723, it can be said that there was a strong correlation.

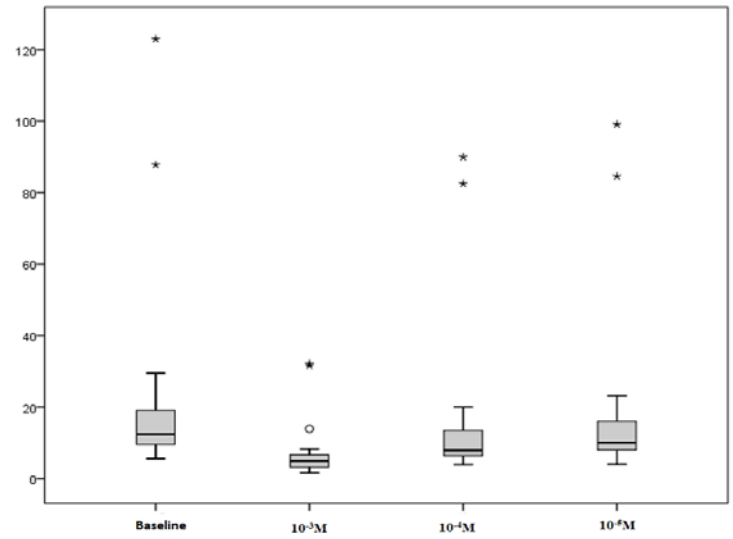

Figure 3. Distribution of urea for urease-GLDH based on the concentrations

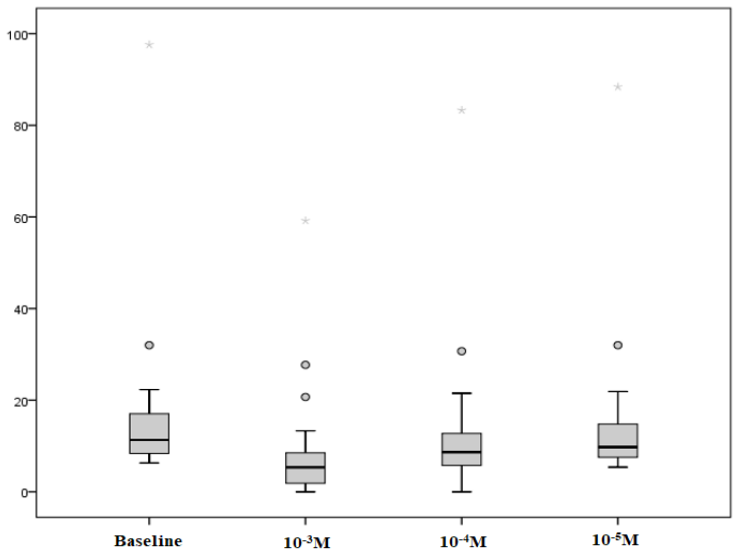

Figure 4. Distribution of urea for nesslerization based on the concentrations

Table 3. Means and mean differences in the urea levels at baseline and 10^{-3} M of glyphosate with 95% CI agreement limits according to the Bland–Altman analyses and with the Pearson correlation coefficients

		Mean difference Mean ± SD	Agreement limits with 95% CI	Spearman's coefficient	Correlation
Urea	Urease-GLDH	1.0 ± 0.3	0.2 to 1.7	0.775	
	Nesslerization	1.0 ± 0.8	–0.4 to 2.7	0.723	

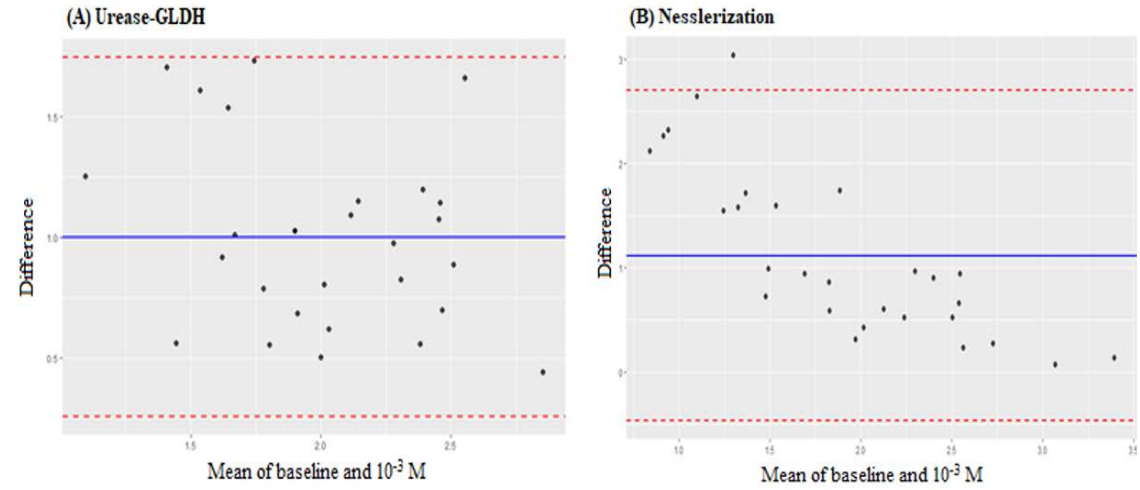

Figure 5. Means, mean differences and agreement limits of urea in urease-GLDH and nesslerization results according to the Bland–Altman analyses. (Straight lines indicate the mean differences of the measurements obtained with the urease-GLDH and nesslerization methods; the dotted lines indicate the 95% CI limits of agreement.)

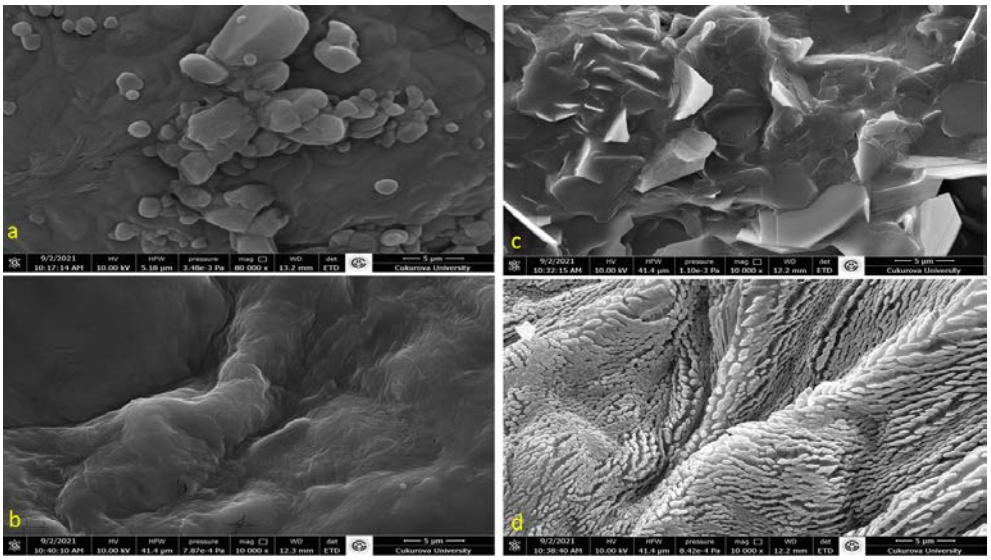

Figure 6. (a) Urease, (b) urease+urea, (c) urease+glyphosate, and (d) urease+glyphosate+urea

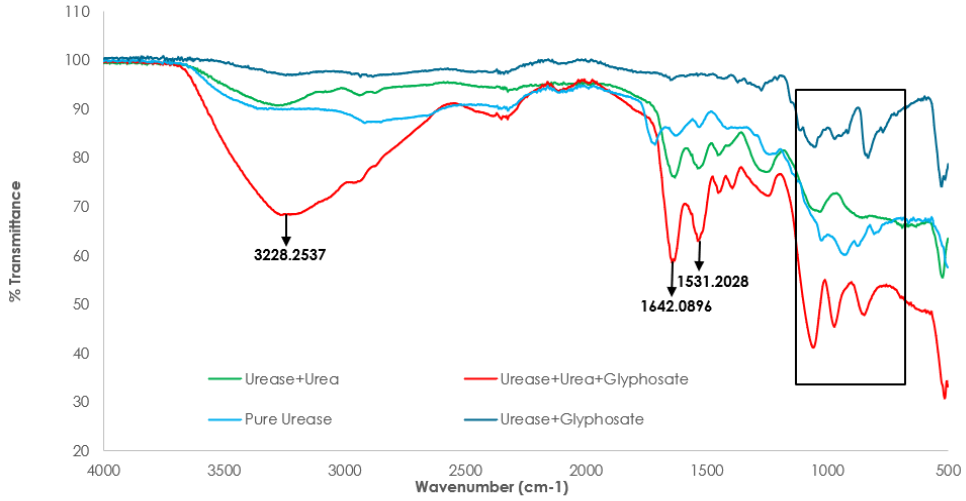

Figure 7. FTIR spectrums of the urease+urea (green line), pure urease (light blue line), urease+urea+glyphosate (red line), and urease+glyphosate (dark blue line)

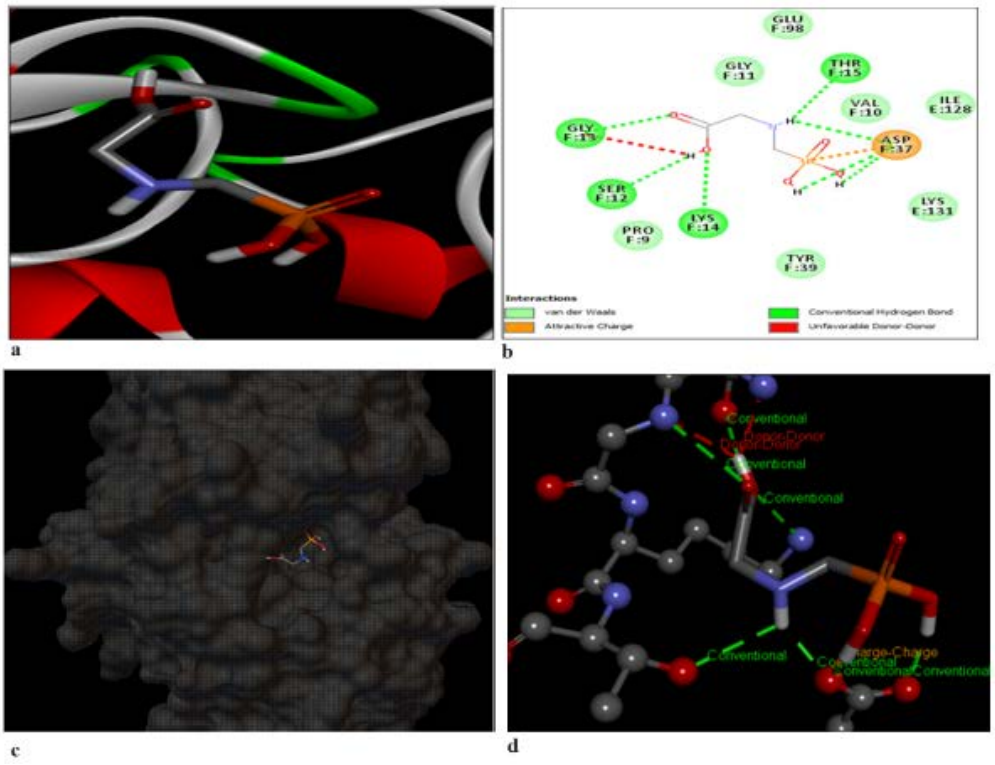

Figure 8. Docking data displaying the interaction between glyphosate and urease (PDB Code: 1BNA). (a) Best docking pose, (b) 2-D interaction, (c) receptor ligand interaction with the H-Bond surface, and (d) solvent hydrogen bond donor/acceptor surface with other bond types.

SEM analysis

Figure 6(a) depicts the pure chemical powder structure of the urease in granular form. When exposed to urease+urea, its granular structure disappeared and was replaced by an amorphous structure (b), and when exposed to glyphosate, indented macrocrystalline structures were created (c). The rough-lamellar form underwent structural modifications after interacting with urease-glyphosate-urea (d).s

FTIR analysis

Figure 7 shows the FTIR spectra in the 4000–500 cm^{-1} range for changes in the chemical structure of the pure urease, urease+urea, urease+glyphosate, and urease+urea+glyphosate sample groups. The interaction of urea and glyphosate resulted in an increase in the transmittance values of the peaks in the 3228.25 (O-H), 1642.08 (C=C), and 1531.20 (N-O) cm^{-1} regions.

These peaks related to the urea+glyphosate interaction were also consistent with the literature[34]. The newly generated bonds were assumed to be responsible for the increase in the transmission values. The transmittance of pure urease was assumed to increase following the interaction of the urea and glyphosate separately in the region near the 1000 cm^{-1} wavelength, but three different peaks in the same region in the presence of both urea and glyphosate were the result of newly formed bonds in the structure.

Molecular docking analysis

In the molecular docking analysis, amino acid interaction and chemical bond types of glyphosate with the urease enzyme based on Gibbs free binding energies (kcal/mol) were investigated (Table 4). As the lowest negative free binding energy (ΔG binding) was –5.24 kcal/mol, it was concluded that the glyphosate interacted with urease near the binding energy threshold. Figure 1 depicts the ideal coupling poses of the glyphosate and urease. It was observed that the glyphosate molecule formed three distinct hydrogen bonds and non-covalent charge-charge interactions with the Asp37 amino acid at the active site of the urease enzyme, as well as a donor-donor bond contact with Gly13 (Figures 8b and 8d).

Table 4. Docking results of the glyphosate and urease complex

Chemical (ligand)	Enzyme (receptor)	ΔG_{Best}	Types of chemical bonds/distance		
			Interaction	Bond type	Distance
Glyphosate	Urease	–5.24 kcal/mol	H16-ASP37	Conventional H-Bond	1.92 Å
			H17-ASP37	Conventional H-Bond	1.84 Å
			H13-ASP37	Conventional H-Bond	2.00 Å
			P1-ASP37	Charge int.	3.27 Å
			H13-THR15	Conventional H-Bond	2.87 Å
			O6-GLY13	Conventional H-Bond	3.05 Å
			H18-GLY13	Donor-Donor	2.58 Å
			H18-SER12	Donor-Donor	2.33 Å
			H18-SER12	Conventional H-Bond	2.34 Å
			O5-LYS14	Conventional H-Bond	2.68 Å

DISCUSSION

In contrast to the rate of global population growth, a lack of production areas and a decrease in arable agricultural areas may lead to food shortages in the coming years. In this regard, sustainable agricultural methods are extremely valuable, and it should be noted that the usage of pesticides (herbicides), which are essential components of contemporary agriculture, is expanding on a daily basis [35]. According to the General Directorate of Food and Control of the Ministry of Agriculture and Forestry in the Republic of Türkiye, a total of 15,021,694 kg L^{-1} plant protection products (insecticide, fungicide, and herbicide) were used in the Mediterranean region in 2020, and 53,672,000 kg L^{-1} plant protection products (insecticide, fungicide, and herbicide) were used throughout Türkiye. Among these products, there has been an increase in the use of the herbicide glyphosate and its derivatives, with the major rationale being to cut production costs [36].

Since humans are omnivores, herbicide residues are detected in the bodies of many people around the world. It is well recognized that herbicides, owing to their permanence effect, stay in the soil and impact both human and animal health through the consumption of agricultural products. This also affect both human and animal health when these chemicals mix with groundwater. For example, organophosphorus insecticides prevent erythrocyte functions by changing the membrane properties of erythrocytes [37–40]. In a previous study, it was found that glyphosate had a negative interference effect on enzyme activity measurements in patients with the common erythrocyte glucose 6-phosphate dehydrogenase enzyme deficiency in the Çukurova region of Türkiye [41].

Interference is a significant source of analytical error in biochemistry laboratories, affecting the study of blood and other body fluids. By indirectly altering patient outcomes, it can lead to misdiagnosis and misguided follow-up, as well as increasing costs through repeated measures [42]. As the objective any biochemistry laboratory is to produce accurate and precise reporting, it is vital that all laboratory personnel and specialists strive to prevent or reduce these types of analytical errors, within the scope of the Quality Control Program [43,44]. There are no published accounts of the interaction between urease and glyphosate at the molecular level. As urease is a nickel metalloenzyme, the current study attempted to understand more about the molecular mechanism underlying glyphosate's influence on this enzyme. The findings have demonstrated that glyphosate negatively affects urea quantification in laboratories employing either one of the two methods, by blocking the urease enzyme. We hypothesize that glyphosate acts on urease via hydrogen bonding (Gly13, Ser12, Lys14, Thr15, and Asp37) and hydrophobic interactions (Val10, Asp37, and Glu98), and that these amino acids may limit the accessibility of the active catalytic conformation of the urease and/or influence the stabilization of the catalytic transition state.

When the molecular docking results were examined, strong hydrogen bond interactions were observed between the glyphosate molecule and amino acid residues Thr15, Gly13, Ser12, and Lys14 in the receptor binding motif of the urease enzyme, while both multiple hydrogen bonds and charge-charge interactions were observed with the Asp37 residue close to the active center of the enzyme. In addition, van der Waals interactions were observed between the glyphosate and residues Glu98, Gly11, and Pro9 in the active center of the enzyme and amino acid residues Val10, Ile128, Lys131, and Tyr39 close to the active center. When the energy levels of all the bonds occurring between the receptor and ligand were examined, it was observed that the interaction occurred with a free binding energy close to the threshold binding energy (–5.24 kcal/mol). In addition, similar findings were obtained in the results of the experimental glyphosate-urease enzyme studies. According to the enzyme activity results, glyphosate inhibited the enzyme activity at a statistically significant level with all the concentrations used, except the lowest dose. Apart from molecular docking analyses, the interaction between the urease enzyme and glyphosate appeared to create physicochemical and biochemical changes in both the SEM analyses and comparison of the FTIR spectra scans.

The limitations of this study were that it was only an in vitro study of glyphosate and the sample consisted of randomly selected waste blood. The sample should be examined in patient groups with low, normal, and high urea values, and its in vivo effect should be investigated in high-risk groups.

In microbiology laboratories, urease tests enable the detection of dangerous pathogenic organisms [45]. Possible patient and/or in vitro exposure to glyphosate is likely to influence and lead to incorrect

results in this laboratory test. While evaluating the urea results in the laboratory by the clinicians, the subject of exposure to glyphosate in the patient's history (especially that of agricultural workers) should be taken into account. However, soil urease tests are conducted in agricultural laboratories, and based on these results, it is believed that urea-fertilized soil will have a high level of microbial activity [46]. In particular, the use of glyphosate and its derivatives as herbicides may lead to incomplete/false results in agricultural applications.

On the other hand, as with *H. pylori*, this impact of glyphosate can result in the formation of less toxic derivatives, and it can be used to target urease-containing bacteria found in humans and animals. A study on the design of quinolones as urease inhibitors revealed that, based on molecular modeling, nickel ions in the active center interact with the carboxylic group present in the structure of the molecule. Due to this interaction, *H. pylori* and *Proteus mirabilis* ureases are inhibited [47–49]. It has been shown that the conversion of hydroxamic acid, hydrazide, and amide into fluoroquinolone carboxylic groups exerts an inhibiting impact [50]. In a separate study reporting the crystal structure of the citrated complex of *S. pasteuri* urease, phosphonate and carboxylate scaffolds were proposed in the modeling of urease inhibitors and deemed to be potent inhibitors [51]. Considering the literature findings, the phosphonic and carboxylic acid groups found in the structure of glyphosate can be tested for urease inhibition once it has been converted to a non-toxic form. Moreover, considering the molecular and biochemical structure interactions of previously published studies, we believe that this study adds a valuable contribution to our knowledge on glyphosate interference.

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