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The interference of baicalein with uric acid detected by the enzymatic method and its correction method

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In recent years, the frequency of clinical application and international recognition of Chinese herbal medicines have been increasing, but the effect of Chinese herbal medicines on common clinical biochemical tests is still unclear. This study aimed to investigate the effect of baicalein, a Chinese herbal medicine ingredient, on uric acid (UA), cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol and to alleviate the interference of baicalein on these assays by improving the reagent. The interferences of baicalein during the detection of these five analytes were investigated on the Hitachi 7600 system. We prepared UA assay kit according to commercial standards to facilitate the improvement of the formulation and evaluated its performance. Tempol, which could eliminate the interference of baicalein, was found based on the chemical properties of the drug, and the optimum concentration for adding it to our UA reagent was determined. We found that the interference was concentration-dependent for five analytes, with the largest negative interference on UA determination. Self-prepared UA assay kit had a safe analysis performance. Our kit and the commercial kit showed a higher interference of - 71.75% and – 89.98% at 200 μg/mL baicalein, respectively. The addition of 5 mmol/L Tempol to the UA reagent could strongly resist the interference of baicalein. In Conclusion, baicalein has a negative interference effect on analysis based on the Trinder reaction, especially UA assay. With the increase in baicalein concentrations, the negative bias increased, and our improved UA reagent could resist the interference of baicalein on UA detection.

Keywords Baicalein, Uric acid, Interference, Trinder reaction

With the accelerated pace of clinical new drug research and development, sometimes we find in our work that test results do not match the clinical symptoms or expectations after the patient has taken the drug. On the one hand, the drug affects the production and metabolism of the measured substances in the patient's body. On the other hand, the presence of the drug or its metabolites in the serum will also interfere with the chemical reactions of some tests, unless avoiding the peak concentration of the drug to collect blood or replacing the test method. It has been shown that some drugs such as calcium dobesilate and etamsylate interfere with creatinine, uric acid (UA), cholesterol, and other biochemical testing based on the Trinder reaction[1,2]. In addition to the currently known drugs that affect the test results, there are often some unknown interactions between drugs and laboratory test results in clinical practice, and the interpretation of these abnormal results poses challenges to clinicians and laboratory staff. In response to this phenomenon, some scholars have attached great importance to it and proposed the application of digitization and automation to the establishment or development of drug-laboratory test interaction databases to reduce clinically incorrect diagnoses and unnecessary treatment[3–6]. However, the information about the combinations of laboratory tests and drugs needs to be constantly updated.

Traditional Chinese herbal medicine is a unique health resource in China. In recent years, as China continues to promote international cooperation and the development of traditional Chinese medicine (TCM), TCM has entered the international vision and shown great potential. Chinese herbal medicine has made significant achievements and progress in the treatment of chronic liver and kidney diseases, leukemia, and solid tumors. However, our understanding of whether Chinese medicine ingredients will interfere with laboratory tests is still shallow. *Scutellaria baicalensis* (also known as Huang-Qin) is one of the commonly used Chinese herbal medicinal ingredients in clinical practice, Chinese people have used the dried root of this medicinal plant for more than 2000 years and it has been listed officially in the Chinese Pharmacopoeia. It is prescribed as a part of a multi-herb formulation, which plays the roles of anti-inflammatory, anti-oxidation, anti-tumor, immune

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Name	Manufacturer
Sodium 3-[Ethyl(m-tolyl)amino]– 2-hydroxy- 1-propanesulfonate Hydrate	Tokyo Chemical Industry (Japan)
Ascorbate oxidase (305 U/mg)	TOYOBO (Japan)
Peroxidase (126 U/mg)	TOYOBO (Japan)
2-[4-(2-Hydroxyethyl)– 1-piperazinyl]ethanesulfonic Acid	Tokyo Chemical Industry (Japan)
Triton X- 100	Aladdin (Shanghai, China)
2-Methylisothiazol- 3(2 H)-one compound with 5-chloro- 2- methylisothiazol- 3(2 H)-one	Aladdin (Shanghai, China)
2-[4-(2-Hydroxyethyl)– 1-piperazinyl]ethanesulfonic Acid	Tokyo Chemical Industry (Japan)
4-Aminoantipyrine	J&K Scientific (Beijing, China)
Uricase (3.70 U/mg)	TOYOBO (Japan)
Baicalein (Product Number: T2721; CAS RN: 491 - 67- 8)	Tokyo Chemical Industry (Japan)
4-Hydroxy- 2,2,6,6-tetramethylpiperidine 1-Oxyl Benzoate Free Radical (Tempol)	Tokyo Chemical Industry (Japan)
Calibrator	Roche (US)
System Calibrator	Beckman (US)
Liquid Assayed Multiqual® Level 1 (694)	Bio-Rad (CA, US)
Liquid Assayed Multiqual® Level 2 (695)	Bio-Rad (CA, US)
Liquid Assayed Multiqual® Level 3 (696)	Bio-Rad (CA, US)
Dimethyl sulfoxide	J&K Scientific (Beijing, China)

Table 1. The materials involved in the experiment.

Test name	Sample volume (μL)	R1 volume (Dilution) (μL)	R2 volume (Dilution) (μL)	Primary wavelength (nm)	Secondary wavelength (nm)
UA (self-prepared)	4.5	150 (0)	50 (0)	600	700
UA	4.2	36 (52)	15 (10)	660	800
CHOL	1.2	18 (72)	–	540	600
HDL-C	1.2	108 (0)	36 (0)	600	700
LDL-C	1.2	108 (0)	36 (0)	600	700
TG	1.6	66 (57)	17 (10)	660	800

Table 2. Test analytical parameters.

regulation, and kidney protection[7,8]. Baicalein, a highly active flavonoid in *Scutellaria baicalensis* extracts, is considered to be the active component of *Scutellaria baicalensis* and its preparations that exert pharmacological effects and have strong antioxidant effects[9,10]. Therefore, we first launched a study on baicalein.

Trinder chromogenic reactions are usually used for the determination of UA, cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Since baicalein has a strong antioxidant effect, theoretically it interferes with the oxidation reaction in vivo or in vitro. UA is a product of purine metabolism in the body, which is mainly excreted by the kidneys. UA testing is usually used to determine the existence of hyperuricemia, and it is of great significance in assessing the therapeutic effect of hyperuricemia. The current clinical laboratory method for determining serum UA is mainly the uricase-peroxidase method, which is susceptible to interference by drug factors. Therefore, in this study, we evaluated the interference of baicalein for the detection of UA, CHOL, TG, HDL-C, and LDL-C using an in vitro addition method, and found an effective anti-interference agent to correct its interference with UA reaction systems, which make herbal medicine more rational and effective in clinical application.

Materials and methods

Reagents and equipment

The materials involved in the experiment are shown in Table 1. All tests were carried out on a Hitachi 7600 automatic analyzer routinely used in hospitals. UA, TC, TG, HDL-C, and LDL-C assay kits are from Beckman, and their analytical parameters are shown in Table 2.

Performance evaluation of self-prepared UA reagent

Control serum from Bio-Rad at the three concentrations were measured in one day by the same person after the calibration, with each sample repeated 15 times. The CV was calculated from the 45 results from one laboratory. Bias was evaluated as the difference between the laboratory's mean measurement value and the target value.

In vitro interference experiments

The in vitro interference test was carried out according to the CLSI EP7-A2 guidelines[11]. 20 mg of baicalein was dissolved in 1000 μL DMSO to make a standard. Then the standard was added to the commercially human-derived quality control serum (Bio-Rad) to prepare the dose-response series. The final baicalein concentrations

for each series were 0, 10, 20, 30, 40, 60, 80, 100, 150 and 200 μg/mL. 100 mg of Tempol was dissolved in 1000 μL DMSO to prepare storage liquid. Then Tempol solution was added to R1 of a self-prepared reagent to prepare the dose-response series. The final Tempol concentrations for each series were 0, 3, 4, 5, 6, 7, and 8 mmol/L. The UA levels were measured using the commercial assay kit (Beckman) and our kit respectively, our reagent formation is shown in Table 3. Specimens were analyzed in triplicate within one analytical run to obtain an average value. An internal quality control (QC) was applied during the experiments to ensure test quality. The degree of interference was measured by percentage deviation.

Statistical analysis

GraphPad Prism version 9.0 and Excel 2016 were used for data analysis and graphing. Data are presented as the mean ± SEM from three independent experiments. The differences between the mean values of normally distributed data were assessed by one-way ANOVA (Dunnett's test). $p < 0.05$ was considered statistically significant, which was indicated by "*", $p < 0.01$ was indicated by "**", $p < 0.001$ was indicated by "***", and $p < 0.0001$ was indicated by "****". The percentage deviations (y-axis) were calculated based on the concentration of the baicalein-free specimen and were plotted vs. the baicalein concentrations (x-axis) or vs. the Tempol concentrations (x-axis).

Results

Baicalein has strong negative interference in uric acid assay

Baicalein is an antioxidant that theoretically reacts with hydrogen peroxide (H_2O_2). The Trinder reaction is a fundamental process employed in a variety of clinical biochemical tests, such as the determination of uric acid (UA), cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein (LDL-C). We inferred that the consumption of H_2O_2 produced in the Trinder reaction by baicalein was the potential mechanism of its interference with these assays. Therefore, the effects of baicalein on five analytes were detected by adding the dose-response series in vitro. Based on the Analytical performance standard for routine analytes in clinical chemistry issued by the National Health Commission of China, the acceptable limits of deviations for the UA, CHOL, TG, HDL-C, and LDL-C were ± 4.5%, ± 4%, ± 5%, ± 8% and ± 8%, respectively. The exogenous addition of baicalein exhibited dose-dependent negative interference with the determination of five analytes (Fig. 1A-F). In the presence of 40 μg/mL baicalein, CHOL, TG, HDL-C, and LDL-C did not exceed the acceptable limits of deviations, with deviations of – 2.48%, – 4.23%, – 7.95%, and – 2.39%, whereas negative interferences of – 29.78% were observed for UA quantification. When the baicalein concentrations reached 60 μg/mL, four of five analytes exceeded the acceptable limits of deviations except for the LDL-C. However, all analytes exceeded the acceptable limits of deviation once the baicalein serum concentration was > 100 μg/mL. These results suggest that baicalein produces significant negative interferences with the determination of five assays based on the Trinder reaction, with the largest negative interference on UA.

(A-E) The effects of the exogenous addition of baicalein on UA, CHOL, TG, HDL-C, and LDL-C quantifications; (F) The percentage deviations of UA, CHOL, TG, HDL-C, and LDL-C.

Preparation and performance evaluation of uric acid assay kit

To better improve the reagents that can resist baicalein interference, we first prepared UA assay kit according to commercial standards and evaluated the performance in accuracy and precision. When distilled water was used as the specimen, the blank absorbance of reagent was below 0.1. According to the Clinical and Laboratory Standards Institute (CLSI) EP15-A2 guidelines[12], the accuracy of the reagent was evaluated by the determination of a known concentration of standard, which showed that the quality requirement of less than ± 5% bias was met (Table 4). Next, the coefficient of variation (CV) of absorbance should be below 3%. The precision evaluation results showed that CV was 0.5% for low-concentration QC (Level 1), 0.5% for Medium-concentration QC (Level 2) and 0.9% for high-concentration QC (Level 3) (Table 5). Finally, a commercial assay kit (Beckman) was applied as a control and specimens were analyzed in triplicate within one analytical run to obtain an average value. Whether using the Beckman reagent or our reagent, the presence of baicalein interfered with the UA detection, and this negative interference shows a dose-dependent manner (Fig. 2). These data suggested a consistent trend between the results by our kit and the commercial kit tested. The interference of 10 μg/mL baicalein on UA assay was – 3.72% by using our reagent, while the interference of 200 μg/mL baicalein reached – 71.75%, also far exceeding the clinically acceptable deviation[13]. Therefore, the presence of baicalein in serum had a significant negative interference on the results of enzymatic determination of UA. The above results suggest that our UA assay kit can perform the next in vitro interference experiments.

R1 (1 L, PH = 6.9)		R2 (1 L, PH = 6.9)	
TOOS (M = 295.33)	0.75 mmol/L	MES (M = 238.3)	75 mmol/L
AOD (305 U/mg)	> 2.5 U/mL	4-AAP (M = 203.24)	0.75 mmol/L
POD (126 U/mg)	> 2.1 U/mL	UOX (3.70 U/mg)	> 0.70 U/mL
MES (M = 238.3)	75 mmol/L	Triton X- 100	0.20%
Triton X- 100	0.20%		
Proclin- 300	0.03%		

Table 3. Our UA reagent formation.

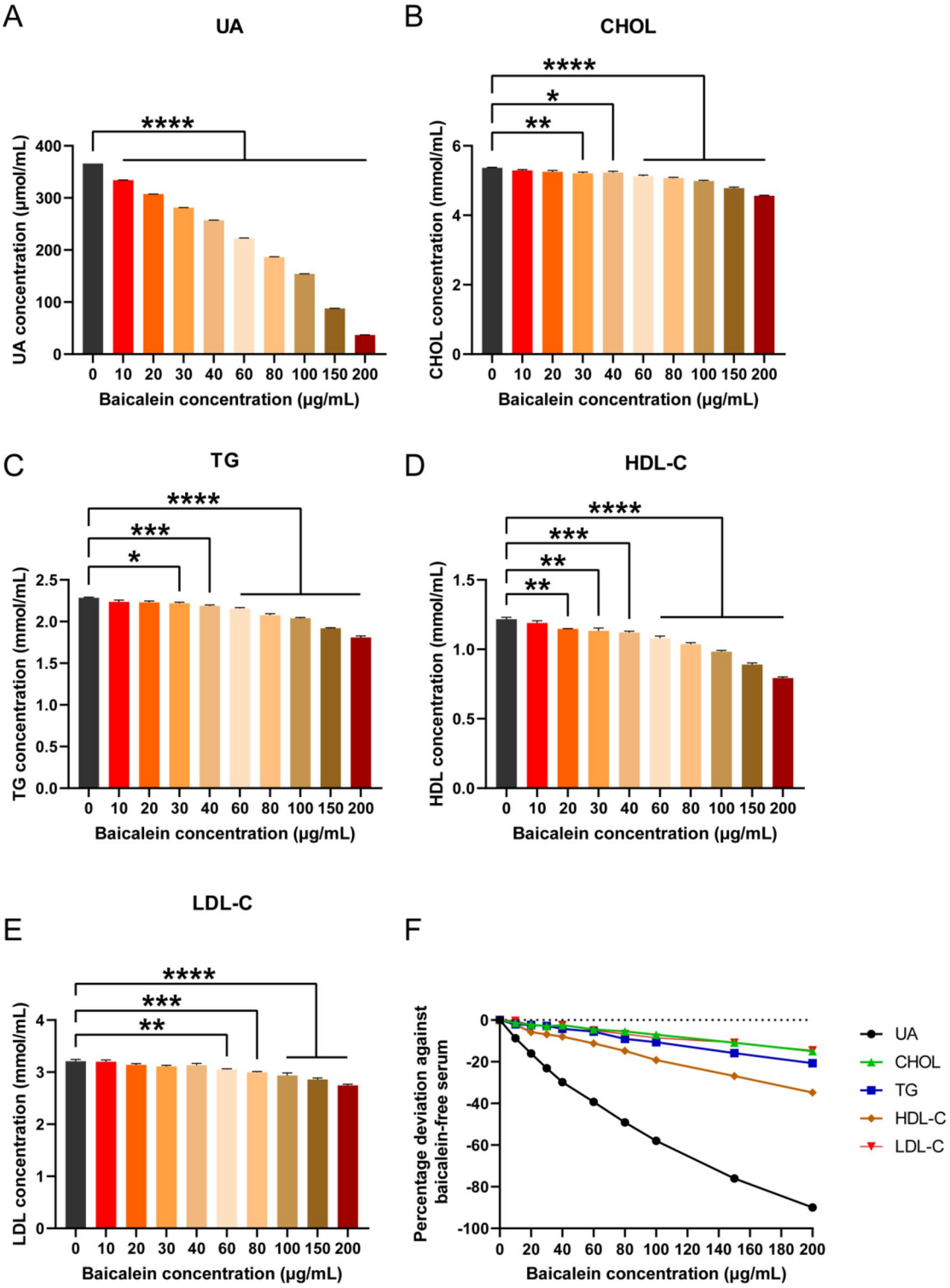

Fig. 1. Effect of baicalein on five analytes.

	Determining level	Estimated value	Bias (%)
UA (μmol/L)	313	313.3	0.09

Table 4. The accuracy evaluation results of our UA assay kit.

Test times	Level 1	Level 2	Level 3
1	231.9	370.4	533.1
2	232.9	374.0	544.1
3	231.9	370.7	549.8
4	233.9	371.9	532.3
5	236.2	370.0	539.3
6	233.9	368.4	532.8
7	233.4	367.9	532.2
8	235.6	371.4	537.1
9	233.0	369.4	535.9
10	233.4	369.2	532.1
11	233.2	368.6	539.3
12	234.3	367.9	538.7
13	233.3	367.6	532.8
14	233.1	372.0	538.1
15	234.8	367.6	539.1
UA mean (μmol/L)	233.7	369.8	537.1
Standard deviation	1.2	1.9	4.9
CV (%)	0.5	0.5	0.9

Table 5. The precision evaluation results of our UA assay kit.

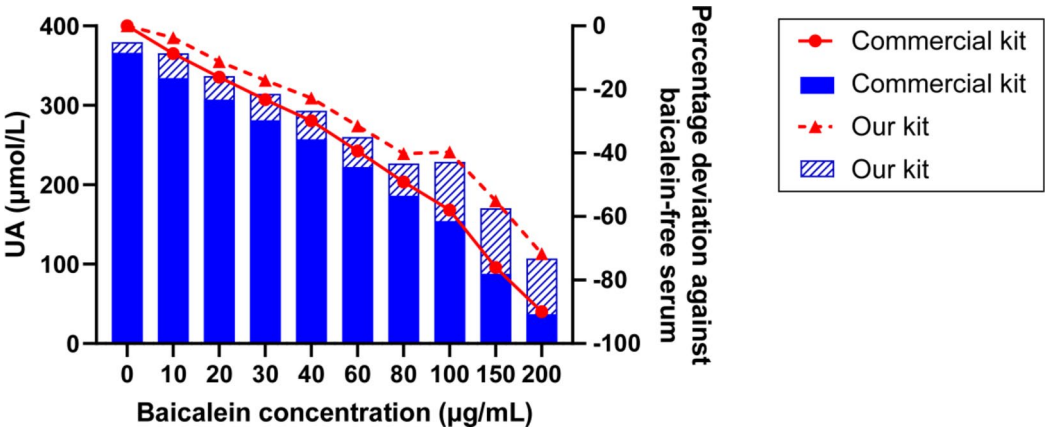

Fig. 2. Effects of baicalein on UA assay with our kit and commercial kit.

The solid blue columns and corresponding red line indicate the uric acid concentrations and baicalein-induced percentage deviations of UA (commercial assay kit), while the hatched blue columns and dashed red line indicate ours.

Tempol could correct the interference of Baicalein on uric acid assay

Tempol, known as 4-hydroxy- 2,2,6,6-tetramethylpiperidine-N-oxyl, is a stable nitrogen oxide radical. We speculate that it can neutralize baicalein in serum and reduce the interference of baicalein with the UA detection system. The ability of Tempol to resist interference by baicalein was detected by establishing a concentration gradient of Tempol, and it was found that the addition of Tempol effectively corrected the negative interference of baicalein on the UA results and the optimal concentration is 5 mmol/L (Fig. 3A, B). What's more, the addition of Tempol in UA reagent in the range of 0.1–10 mmol/L has less than 3% interference on uric acid detection, which meets the technical requirements of UA assay kit (Table 6). Finally, our UA assay kit (with Tempol) was prepared and compared the anti-interference capacity against baicalein with two commercial kits. Considering the clinically acceptable deviation of ± 4.5% for UA, the experimental data conclusively demonstrate that our optimized uric acid detection kit exhibits significant resistance to baicalein-induced interference (Table 7).

Percentage deviations were used to evaluate the resistance of different concentrations of Tempol to baicalein interference. (A) and (B) employ identical experimental methodologies, with (B) specifically demonstrating the impact of low-concentration Tempol supplementation on the anti-interference capacity of uric acid assay reagents.

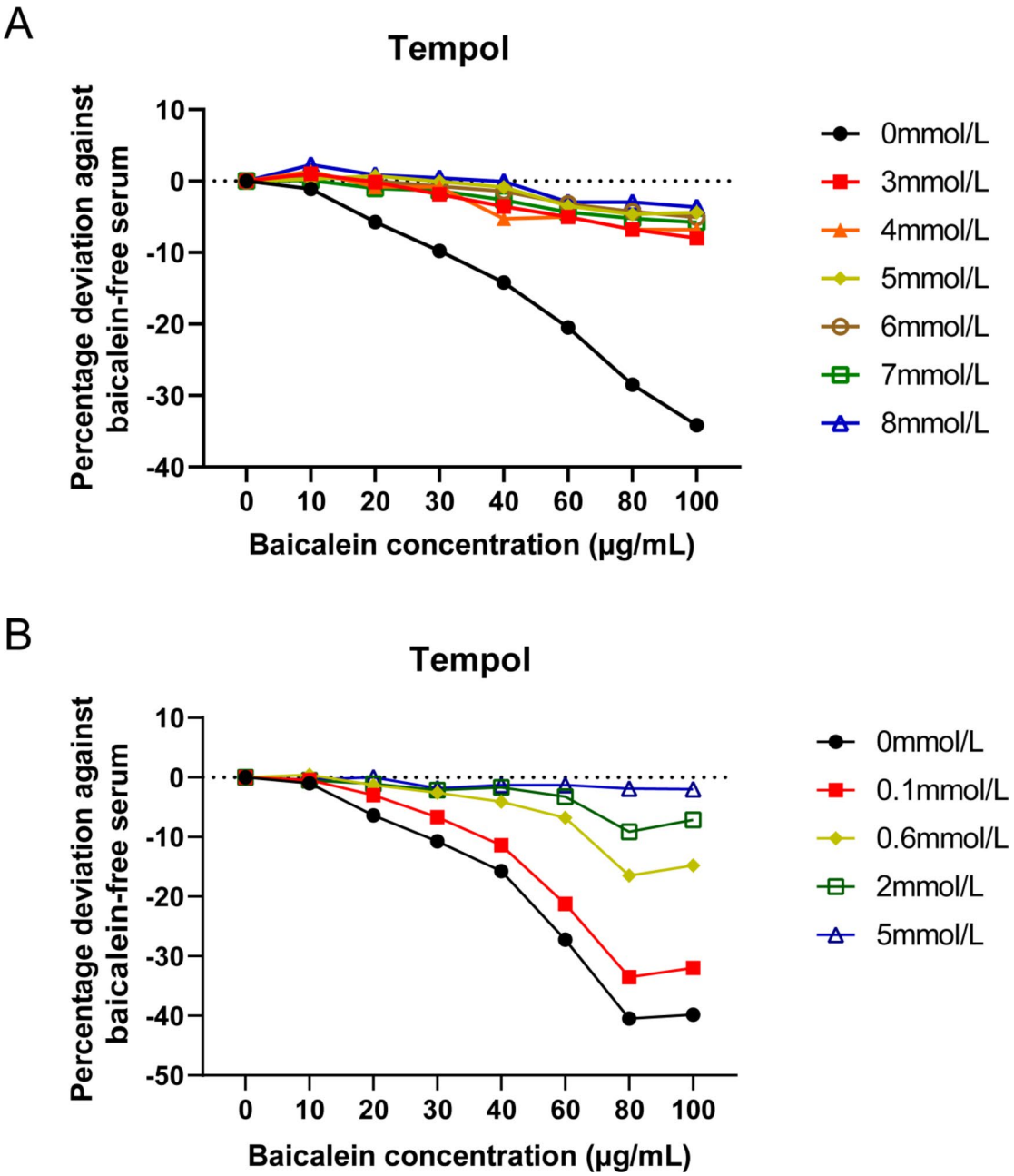

Fig. 3. Tempol corrected the interference of baicalein on UA assays.

Tempol (mmol/L)	0	0.1	0.2	0.3	0.4	0.5	0.8	1	2	5	10
UA (μmol/L)	365.3	365.1	365	365.3	365.6	366.6	366.4	365.6	367.2	370.2	368.6
Bias (%)	–	– 0.05	– 0.08	0.00	0.08	0.36	0.30	0.08	0.52	1.34	0.90

Table 6. Effect of tempol on uric acid detection.

Discussion

In this study, we investigated the interference effects of baicalein on five Trinder reaction-based biochemical assays: CHOL, TG, HDL-C, LDL-C, and UA. The data revealed a pronounced, concentration-dependent interference pattern specifically in uric acid quantification, with maximal interference reaching 89.98% ($p< 0.001$) at 200 μg/mL baicalein. The Trinder reaction, a cornerstone in clinical biochemistry, operates through the enzymatic generation of H_2O_2, which subsequently reacts with 4-aminoantipyrine (4-AAP) and peroxidase (POD) to form a red quinoneimine chromogen. While our study focused on baicalein's interference with

Baicalein(μg/mL)	0	10	20	30	40	60	80	100
Our kit	365.6	356.1	358.9	365.5	370	359	344	325.6
Bias (%)	–	− 2.59	− 1.83	− 0.027	1.20	− 1.8	− 5.9	− 10.9
Commercial kit A	365.3	334.2	304	278.9	258	221.4	184.8	155.4
Bias (%)	–	− 8.51	− 16.78	− 23.65	− 29.37	− 39.39	− 49.41	− 57.4
P value [a]	0.7565	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Commercial kit B	365.1	333.1	306	280.1	259.2	223.2	186.3	157.2
Bias (%)	–	− 8.76	− 16.18	− 23.28	− 29.01	− 38.86	− 48.97	− 56.94
P value [b]	0.7022	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 7. Comparison of anti-interference capacity of our kit and commercial kits. [a] Comparison of our kit and commercial kit A. [b] Comparison of our kit and commercial kit B.

five Trinder-based assays (CHOL, TG, HDL-C, LDL-C, UA), the Trinder reaction is also used for creatinine detection via the sarcosine-oxidase method. In this process: Creatinine → Creatine (creatinase); Creatine → Sarcosine + Urea (creatininase); Sarcosine → Glycine + Formaldehyde + H_2O_2 (sarcosine oxidase); H_2O_2 + 4-AAP + POD → Quinoneimine (proportional to creatinine). The formation of H_2O_2 suggests analogous interference in creatinine assays. Baicalein, a phytochemically purified flavonoid, has been extensively characterized for its pleiotropic bioactivities, including anti-inflammatory, antimicrobial, antitumor, antioxidant, and neuroprotective properties[14,15]. In recent years, there has been much literature on the antioxidant properties and clinical applications of baicalein, but it is not clear whether baicalein will interfere with some common testing based on the principle of redox reaction for determining the level of small molecules in the body. It has been found that taking some drugs can interfere with the detection of creatinine, UA, and other substances, which means that it is of great significance to continuously clarify the phenomenon of drug interference and eliminate drug interference in advance to ensure the accuracy of the test results[1,16]. Our results indicate that the baicalein pharmacological activity raises critical concerns regarding its interference with clinical uric acid assays. In addition, baicalein can reduce UA production and promote UA excretion by inhibiting the activity of xanthine oxidase, baicalein-induced suppression of serum UA levels may underestimate true pathological urate burden, potentially leading to misinterpretation of therapeutic efficacy in clinical practice[17,18].

According to the pharmacokinetics of baicalein, the main metabolites present in the plasma of orally administered baicalein after it is metabolized are baicalein-O-diglucuronide and baicalein- 7-O-sulfate[19–23]. Current research on baicalein pharmacokinetics in humans remains limited, with existing studies predominantly conducted under controlled experimental conditions. Notably, baicalein is rarely administered as a monotherapy in traditional Chinese medical practice, where it is typically combined with other herbal constituents in polyherbal formulations. This necessitates explicit consideration of potential herb-herb interactions. Furthermore, baicalein undergoes extensive hepatic metabolism in humans, yielding elevated systemic levels of metabolites containing catechol-like moieties that retain significant antioxidant capacity. Additionally, some research demonstrated that concomitant food intake substantially enhances the plasma concentrations of both baicalein and its bioactive metabolites[20]. Based on the complicated biotransformation of baicalein in vivo, we added a higher concentration of baicalein in vitro. Our results conclusively demonstrate that the optimized uric acid assay effectively neutralizes baicalein-induced analytical interference, achieving clinically acceptable accuracy even at pharmacologically exaggerated serum concentrations of baicalein (80 μg/mL). This interference-suppression capability confirmes the assay's diagnostic reliability under extreme drug exposure conditions.

Baicalein is also known as 3-hydroxyflavone (5,6,7-trihydroxyflavone), and its antioxidant activity is closely related to the structure of the hydroxyl group in the molecule. The phenolic hydroxyl group removes the hydrogen atoms and reacts with the free radical to form a resonance-stabilized semiquinone radical structure after eliminating the free radicals, which is attributed to the fact that the phenoxy radical forms an intramolecular hydrogen bond with the ortho-hydroxyl group to maintain the stability of the radical structure. The bond dissociation enthalpy (BDE) of hydroxyl groups at different positions on the baicalein molecule was calculated by density functional theory, and 6-OH was found to be the most reactive and strongest active site[24]. In general, baicalein eliminates free radicals by hydrogen atom transfer (HAT), the theoretical parameter of which is the BDE that measures the energy required for O-H bond cleavage. However, in polar solvents, the reaction mechanism favors concerted electron-proton transfer, where the solvent molecule is a hydrogen bond acceptor, and intermolecular hydrogen bonds can be formed between phenol hydroxyl groups and the solvent molecule, thus hindering hydrogen abstraction, and greatly reducing the rate of free radicals scavenging by flavonoids via hydrogen abstraction reactions[25,26]. Building upon the chemical properties of baicalein, we demonstrate that the incorporation of the stable nitroxide radical Tempol into UA assay reagents effectively mitigates baicalein-induced analytical interference. Mechanistically, Tempol functions as a selective electron transfer mediator, neutralizing baicalein's ortho-diphenolic hydroxyl groups' capacity to scavenge H_2O_2 - a critical intermediate in Trinder's reaction. Tempol is considered a therapeutic antioxidant because of its superoxide dismutase-like activity, which scavenges free radicals, promotes reactive oxygen species (ROS) metabolism, and protects mitochondria from oxidative damage in organisms[27]. For example, Tempol treatment causes a reduction in oxidative stress and improves lipid profile in mice[28,29]. Secondly, as a radical, Tempol has unpaired single electrons, which can be oxidized or reduced through the gain or loss of electrons, so it can be used as a contrast agent. Because the concentration of endogenous paramagnetic substances (e.g. paramagnetic metal ions and radicals)

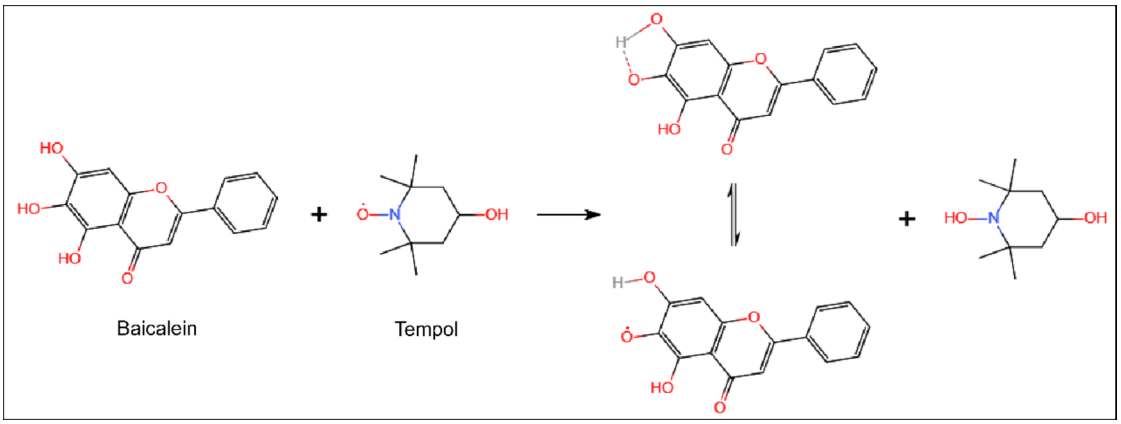

Fig. 4. The reaction of baicalein with Tempol.

is not sufficient to be directly detected by electron paramagnetic resonance (EPR) in vivo, exogenous stable radicals (e.g. paramagnetic nitroxides) have to be introduced as probes. Tempol can be reduced to diamagnetic hydroxylamine in the hypoxic site, and the redox state in vivo can be detected by detecting the ratio of the two[30–32]. Rímal et al.[33] found that the addition of ascorbic acid to the solution effectively scavenged Tempol and generated Tempol-H and an ascorbyl radical, a reaction mediated by HAT. May et al.[34] also found that Tempol can interact with glutathione and ascorbic acid, the main antioxidants in endothelial cells. Therefore, we believe that the properties of Tempol radical can be used to eliminate the interference of antioxidant baicalein in the UA assay. Currently, the principle of the UA detection method applied in clinical laboratories is that UA is converted into allantoin and H_2O_2 under the action of uricase, and H_2O_2 is catalyzed by peroxidase to form quinone imide compounds with 4-AAP and TOOS, its production amount is proportional to the concentration of UA. We speculate that baicalein in human serum might be consuming the intermediate product H_2O_2 to falsely reduce the UA results, and our results show that Tempol does not interfere with these two chemical reactions while eliminating baicalein. The results of Hahn et al.[35] also showed that Tempol does not consume H_2O_2. Therefore, we added a certain concentration of Tempol to R1 of UA assay kit to consume baicalein before the official start of UA detection to ensure the correctness of the amount of chromogenic substrate. The two substances may react through the HAT mechanism and mainly occur in the 6-OH of baicalein, as shown in Fig. 4[33,36].

The QC of laboratory testing mainly includes three aspects: pre-analytical quality assurance, QC during analysis, and post-analytical quality evaluation. Among these three aspects, pre-analysis quality assurance is the most difficult to control, with many influencing factors and the largest variables. Drug interference is one of the influencing factors. For example, ascorbate oxidase has been added to many reagents to reduce the interference of vitamin C on the results of biochemical testing. Vitamin C can reduce the intermediate product H_2O_2 and the final product generated in the process of UA determination, thus resulting in the false reduction of serum UA results. Currently, there is no authoritative statistical data on the exact number of people in China who consume *Scutellaria baicalensis*. However, as a key component in traditional Chinese medicine and modern compound formulations, it is widely used among the population for treating various diseases, such as respiratory and digestive system disorders, as well as infectious diseases. With ongoing advancements in anti-tumor and anti-viral research, its clinical applications are expected to expand further in the future. We found that baicalein causes negative interference in the detection of UA by the uricase-peroxidase method. The addition of stable radical Tempol to the UA reagent can resist the interference of baicalein in the testing process of UA, provide more accurate clinical test results, and guide the clinical application of baicalein and similar drugs with antioxidant effects. Our current study has several limitations. First, while we validated Tempol's interference-suppression efficacy in UA quantification, its applicability to the other four Trinder's reaction-based assays (CHOL, TG, HDL-C, LDL-C) remains unverified. Second, the experimental design focused exclusively on pure baicalein, without accounting for potential synergistic interference effects from its major metabolites (e.g., baicalein-O-diglucuronide and baicalein- 7-O-sulfate) that circulate in treated patients. Third, we may not fully replicate the complex biomatrix of patients undergoing baicalein-containing therapy, where concurrent medications and individual metabolic variations could modulate interference dynamics.

Conclusion

This study systematically validated the negative interference of baicalein on Trinder's reaction-based clinical assays by adding a gradient concentration of baicalein (0–200 μg/mL) to human serum. The results revealed that uric acid quantification exhibited the most pronounced susceptibility, demonstrating a dose-dependent interference up to − 89.98% deviation at maximal concentration. To address this pharmacological interference, we developed an optimized uric acid assay incorporating 5 mmol/L Tempol as a selective antioxidant scavenger, which effectively neutralized baicalein-induced interference. It provides a way to solve the problem that taking drugs with antioxidant properties can interfere with clinical test results.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

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