

World Journal of *Pharmacology*

World J Pharmacol 2023 February 17; 12(1): 1-11



ORIGINAL ARTICLE**Randomized Clinical Trial**

- 1 Pharmacokinetics/Pharmacodynamics study of Fixtral SB as compared to supra bioavailable itraconazole and conventional itraconazole

Naqvi SMH, Gala MYN, Muchhala S, Arumugam A, Panigrahi D, Patil D, Rathod R, Mane A

ABOUT COVER

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INDEXING/ABSTRACTING

The WJP is now abstracted and indexed in Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Si Zhao; Production Department Director: Xiang Li; Editorial Office Director: Yun-Xiaojiao Wu.

NAME OF JOURNAL

World Journal of Pharmacology

ISSN

ISSN 2220-3192 (online)

LAUNCH DATE

February 9, 2012

FREQUENCY

Continuous Publication

EDITORS-IN-CHIEF

Muhammad Shahzad, Ahmed M Kabel, Pharkphoom Panichayupakaranant

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2220-3192/editorialboard.htm>

PUBLICATION DATE

February 17, 2023

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INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Randomized Clinical Trial

Pharmacokinetics/Pharmacodynamics study of Fixtral SB as compared to supra bioavailable itraconazole and conventional itraconazole

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Specialty type: Pharmacology and pharmacy

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Norman TR, Australia; Trkulja V, Croatia

Received: September 16, 2022

Peer-review started: September 16, 2022

First decision: October 31, 2022

Revised: November 11, 2022

Accepted: February 7, 2023

Article in press: February 7, 2023

Published online: February 17, 2023



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Abstract

BACKGROUND

Itraconazole is a broad-spectrum triazole antifungal inhibiting fungal growth by inhibiting ergosterol synthesis and exhibits a nonlinear pharmacokinetic profile. Erratic absorption pattern with wide fluctuations in blood levels causes inconsistent and unpredictable clinical behaviour of this drug despite its low minimum inhibitory concentration (MIC) as compared to other antifungal agents.

AIM

To compare the oral bioavailability and bioequivalence of Fixtral SB (supra bioavailable itraconazole) with reference product R2 (supra bioavailable 2 × 50 mg itraconazole).

METHODS

The study population consisted of 54 healthy volunteers, aged between 18-45 years and randomized to receive a single oral dose of either test [T; Fixtral SB (supra bioavailable itraconazole) 100 mg] or reference product (R1; Sporanox 100 mg × 2 capsules and R2; Lozanoc capsules 50 mg × 2 capsules). Blood samples were taken pre-dose and post-dose up to 96 h. The study evaluated bioequivalence by comparing the oral bioavailability of the test product with reference product R2. The pharmacodynamic characteristics of the drug were evaluated by comparing the test product with reference product R1. Pharmacokinetics (PK)-PD

comparative analysis [area under the concentration-time curve (AUC)/ minimum inhibitory concentration (MIC) > 25] was performed for conventional itraconazole 100 mg and supra bioavailable itraconazole 50 mg. Adverse events (AEs) assessments were performed in each study period and post-study evaluation.

RESULTS

Statistical analysis of primary PK variables revealed bioequivalence, with confidence intervals being completely inside the acceptance criteria of 80%-125%. The peak concentration levels of itraconazole were achieved at 10 h (T) and 8.5 h (R2), respectively. Pharmacodynamic parameter assessment showed that AUC/MIC for R1 are comparable to Fixtral SB 100mg for MIC levels up to 16mcg/mL ($P > 0.05$ and observed $P = 0.3196$). Six AEs were observed that were mild to moderate in severity and resolved. No severe AE was reported.

CONCLUSION

Test product itraconazole Capsule 100 mg is bioequivalent with the reference product (R2) at 100 mg dose (2 capsules of Lozanoc® 50 mg) under fed conditions. Pharmacodynamics activity in terms of AUC/MIC is comparable between the test product at 100 mg dose and marketed itraconazole 200 mg. Fixtral SB is expected to have therapeutically similar efficacy at half the equivalent dose. Tested formulations were found to be safe and well tolerated.

Key Words: Supra-Bioavailable itraconazole; Conventional itraconazole; Oral bioavailability; Pharmacodynamics; Efficacy; Adverse events

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Core Tip: Itraconazole, a triazole antifungal with a broad spectrum, has a nonlinear pharmacokinetic profile due to its variable oral bioavailability. Based on these criteria, a comparison of Fixtral SB (supra bioavailable itraconazole) 100 mg with Lozanoc capsules 50mg administered as two capsules was performed, revealing that Fixtral SB is expected to have therapeutically comparable efficacy at half the equivalent dose. In terms of area under the concentration-time curve/minimum inhibitory concentration, the pharmacodynamic activity of the test product at 100 mg dose and the marketed itraconazole 200 mg is comparable. All of the formulations tested were found to be safe and well tolerated, with manageable side effects.

Citation: Naqvi SMH, Gala MYN, Muchhala S, Arumugam A, Panigrahi D, Patil D, Rathod R, Mane A. Pharmacokinetics/Pharmacodynamics study of Fixtral SB as compared to supra bioavailable itraconazole and conventional itraconazole. *World J Pharmacol* 2023; 12(1): 1-11

URL: <https://www.wjgnet.com/2220-3192/full/v12/i1/1.htm>

DOI: <https://dx.doi.org/10.5497/wjp.v12.i1.1>

INTRODUCTION

Itraconazole is a broad-spectrum triazole antifungal with favorable pharmacokinetic profile[1]. It is an effective and well tolerated medication for superficial and invasive systemic fungal infections, including aspergillus species. Anti-fungal efficacy of its active metabolite 'hydroxyitraconazole is double to that of parent compound[1,2]. It inhibits the fungal growth by inhibiting ergosterol synthesis, a major component of fungal cell membrane by inhibiting the enzyme 14 α sterol demethylase - a microsomal cytochrome P450 enzyme, responsible for catalytic conversion of lanosterol to ergosterol[3,4].

Itraconazole exhibits a nonlinear pharmacokinetic profile due to its varied oral bioavailability[5-7]. It has prolonged terminal clearance time, biphasic elimination with a terminal half-life of roughly 24 h and reaches a steady state in 14 days[4]. Its lipophilic and weakly basic nature renders it water-insoluble, representing a rate-limiting step in its absorption in the gastrointestinal tract[4,8]. Food has an impact on the bioavailability of itraconazole as maximum absolute bioavailability (55%) is achieved in fed state[8]. It is metabolized in the liver by CYP3A4 enzyme to its active metabolite hydroxyitraconazole which is 99% bound to plasma proteins[4]. Metabolites are excreted through bile and urine, only 3%-18% are detected in feces[1,2].

Several studies determining the effect of food on itraconazole absorption showed that it is highly varied between subjects, and the measured area under the concentration-time curve (AUC) values in the fed and fasted states have shown significant interstudy variability[9,10]. Erratic absorption pattern with

wide fluctuations in blood levels causes inconsistent and unpredictable clinical behavior of this drug in spite of its low minimum inhibitory concentration (MIC) as compared to other antifungal agents[11]. Drug interactions leading to enhanced metabolism or reduced bioavailability can also result in reduced efficacy of itraconazole[12].

Itraconazole is widely used worldwide for systemic as well as local fungal infections with different dosage regimens depending on the site of infection. Dosages from 100 mg/day to 600 mg/day of conventional itraconazole are approved for oropharyngeal esophageal candidiasis, systematic aspergillosis, candidiasis, cryptococcosis, sporotrichosis, blastomycosis and other rarely occurring systemic or tropical mycosis[13].

Test product Fixtral SB has been manufactured *via* APSG™ (Advanced Polymeric Spray Granulation), a breakthrough innovative proprietary technology for enhancing solubility and maximizing bioavailability of poorly soluble drug itraconazole as depicted in Figure 1 and Figure 2. Conventional itraconazole formulations show complete drug release in acidic pH (stomach) and minimum drug release in basic pH (Intestine). Though intestine is a good site of absorption, but as soon as itraconazole from conventional formulations enters into the small intestine, the change in pH causes the itraconazole to precipitate which was dissolved in stomach. Therefore, the change in pH due to gastric emptying and ingestion of food significantly affects the dissolution of itraconazole from conventional formulations and there by decreases bioavailability and increases variability in absorption. To overcome the limitations of conventional itraconazole formulations, APSG™ technology as depicted in Figure 1 and Figure 2 was adopted to produce amorphous solid dispersion of itraconazole by using an enteric polymer for enhancing the bioavailability. Enteric polymer is chosen over all other polymers owing to its optimum & rapid solubility in intestinal pH and ability to restrict the drug release in acidic environment which minimizes the precipitation potential of itraconazole in intestine thus maximizing bioavailability. The drug and polymer ratio has been optimized to get an optimum concentration of polymer in the formulation to provide a stable amorphous solid dispersion throughout the shelf life.

Based on this technology with improved bioavailability, itraconazole 50 mg is approved in Australia and India[4].

Pharmacokinetics (PK) parameters (C_{max} , T_{max} , AUC) demonstrate drug exposure in humans and pharmacodynamic parameters are derived using these conventional PK parameters. Various pharmacodynamic parameters are used to determine antifungal activity of drugs such as C_{max}/MIC , $T > MIC$, AUC/MIC depending on concentration dependent or/and time dependent mechanism of action. For triazoles, AUC/MIC is an appropriate PD parameter to demonstrate antimicrobial activity as these have extensive post antifungal effect[14]. For efficacy with 50% maximal effect, AUC/MIC value around 25 is reported for triazole class[14].

In PK-PD comparative study of conventional itraconazole 100 mg and supra bioavailable itraconazole 50 mg, AUC/MIC > 25 was evaluated. Comparing AUC/MIC ratio for both treatment arms, it was proposed that conventional itraconazole 100 mg is expected to have comparable efficacy at half dose *i.e.*, 50 mg of the novel formulation and approved at half the dose[15]. Additionally target AUC/MIC 25-50 for optimal efficacy for triazole class of antifungal was reported[14,16,17].

The main objective of this study was to compare the oral bioavailability of Fixtral SB (supra bioavailable itraconazole) 100 mg (test product; Dr. Reddy's Laboratories Limited) with Lozanoc capsules 50 mg administered as 2 capsules (reference product (R2; Mayne Pharma International Pty Ltd), with equivalence for AUC parameter, in order to determine the bioequivalence of both the formulations. The secondary objective was to compare the pharmacodynamics/ characteristics of test product with Sporanox 100 mg × 2 capsules (reference product (R1; Johnson & Johnson Private Limited) and lastly to monitor the safety and tolerability of the study drugs.

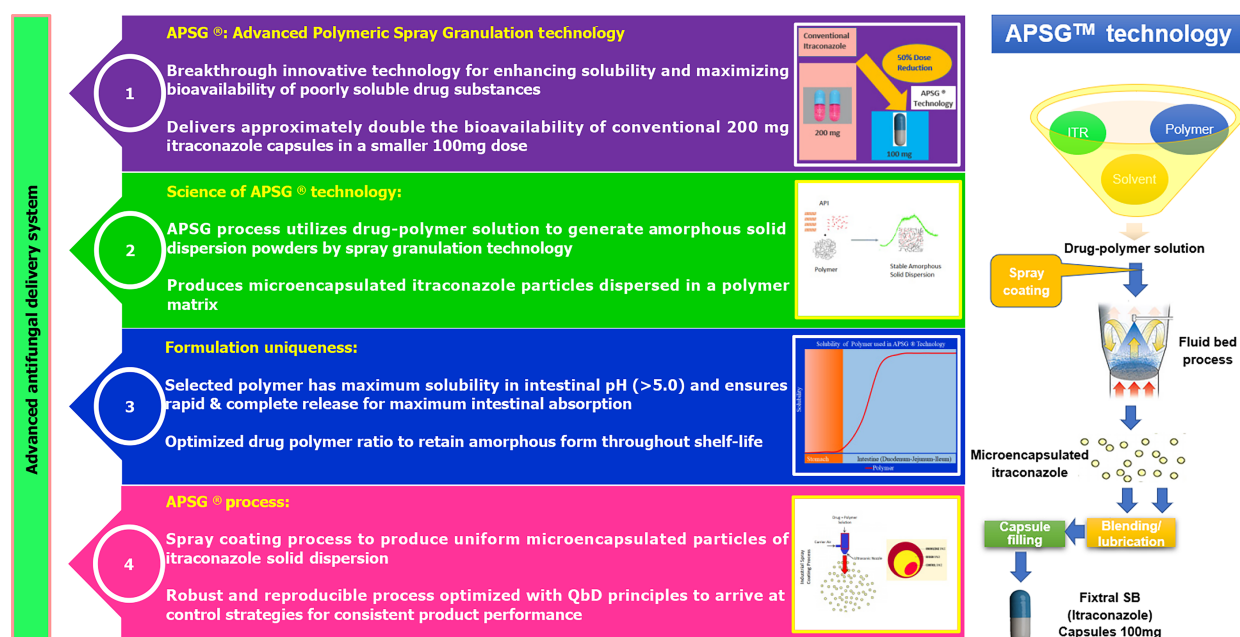
MATERIALS AND METHODS

Study design and setting

This was a single dose, randomized, three treatments, three period, three sequence, open label, balanced, crossover, oral comparative bioavailability study conducted at Accutest Research Laboratories (I) Pvt. Ltd, Maharashtra, India from 19 July 2021 to 27 August 2021. The study protocol, informed consent forms and other relevant documents (subjects' accrual) were reviewed and approved by 'Ethicare Ethics committee'. Pre-screening and general safety instructions in view of COVID-19 pandemic were reviewed and approved by 'Human Care Independent Ethics Committee'. The study was performed in compliance with the principles of the Declaration of Helsinki for Medical Research involving human subjects, the Guideline for Good Clinical Practice (GCP) and the Drug Control General of India (DCGI) regulatory guidelines. All volunteers provided their written informed consent to participate before executing any study-related procedure. Study design and subjects' disposition is presented in Figure 3.

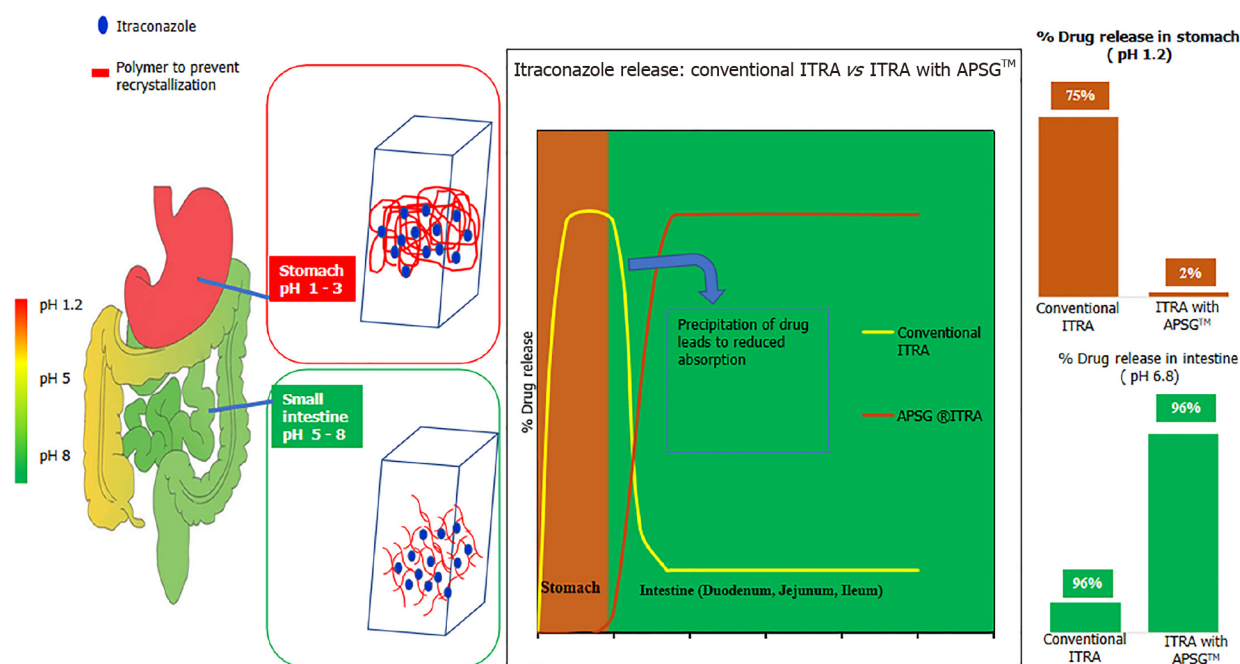
Study drugs

Supra bioavailable itraconazole capsule, Fixtral SB 100 mg, (batch number CV21002) manufactured by



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Figure 1 Itraconazole with APSG™: Maximizing bioavailability.



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Figure 2 Conventional itraconazole vs APSG™ itraconazole.

Dr. Reddy's Laboratories Limited, India was used as the test product. Two reference products were Sporanox® capsules 2 × 100 mg (batch number N192) manufactured by Johnson & Johnson Private Limited, Mulund, Mumbai as reference product (R1) and Lozanoc capsules® 2 × 50 mg manufactured by Mayne pharma International pty Ltd, Salisbury South, South Australia, as reference product (R2).

Subjects

The study population consisted of healthy male and female volunteers, aged between 18-45 years. Participants with a body mass index (BMI) of 18.5-30 kg/m² and a weight of ≥ 45 kg, non-pregnant, non-smokers, ex-smokers or moderate smokers with no history of drug abuse and significant alcoholism were eligible for enrolment into the study.

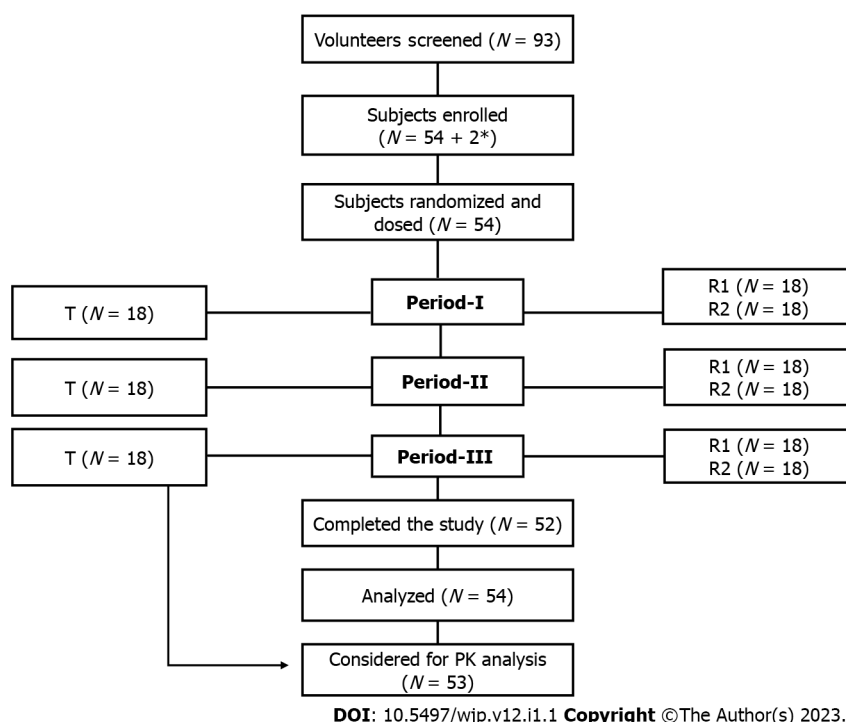


Figure 3 Flowchart of study design and subject disposition. PK: Pharmacokinetics. *2 were the additional subjects which were replaced with the subjects withdrawn due to adverse events.

Subjects found to be healthy during pre-study screening/ evaluation (*i.e.*, 21 days prior to dosing of period-I) after physical examination, vital sign assessment, ECG examination and other clinical laboratory analysis (haemogram, biochemistry, serum electrolytes, urinalysis) were included in the study.

Volunteers with clinically relevant abnormalities in medical examination/ history, or having any systemic disease (cardiovascular, renal, metabolic, gastrointestinal, haematological, *etc.*) medical or surgical condition interfering with the functioning of gastrointestinal tract were excluded. In addition, individuals with a known history of hypersensitivity to itraconazole and other related formulations; or requiring medication for an ailment having enzyme modifying activity (previous 28 days prior to dosing day) or using prescription medication (14 days prior to dose administration); participation in other bioequivalence study or blood donation (90 days prior to period-I dosing); positive for drug or alcohol abuse; positive for HIV, HbsAg or Hepatitis -C tests; pregnant; use of hormonal contraceptives or lactating women were not included.

Study procedure

A randomized, open label, balanced, three treatments, three period, three sequence, crossover, oral comparative bioavailability study was carried out in healthy male and female human volunteers under fed conditions. A total of 92 volunteers were screened, of these 54 subjects were included in the study. Subjects were housed for at least 10.50 h prior to study drug administration until 24 h post dose in each study period. Subjects were randomized to receive single oral dose of either the test product (T) or reference product (R1 and R2). Duration of treatment was 39 days including a washout period of 14 days between period I and period II and 20 days between period II and period III. After an overnight fasting of 10 h, each subject was given high fat and high calorie breakfast 30 minutes prior to the drug administration. Study subjects were given 240 mL of water at ambient temperature in sitting position. Water was restricted for 01 h pre-dose to 01 h post-dose except fluid that was taken during breakfast and 240 mL at the time of study drug administration in each study period. Subjects were asked to remain awake and in upright position up to 4 h. post dose, except for any procedural reason or in case of an adverse event. Standardized meals were given during check in night, 04, 09 and 13 h post dose in each study period. Blood samples were taken pre-dose (00 h) and post-dose up to 96 h. Samples after 24 h were collected on an ambulatory basis.

Blood sampling

A total of 21 blood samples (5 mL/ sample) were collected in K2- ethylenediaminetetraacetic acid (EDTA) vacutainers. Approximately, 345.2 mL of total blood was withdrawn from each subject. Samples were taken pre-dose (00 h prior to dosing), and at 01, 02, 02, 03, 04, 04.50, 05, 05.50, 06, 06.50, 07, 08, 09, 10, 12, 24, 36, 48, 72 and 96 h post-dose. Following centrifugation under refrigeration at 3500 rpm for 10

minutes at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$, the plasma was transferred to appropriate size biological sample storage vials (previously labeled with study code and sample code), in duplicate (one aliquot as control samples and one aliquot for analysis; the aliquot for analysis was contained approximately 1.5 mL of plasma and remaining approximately 0.5 mL of plasma was transferred in control aliquot).

Buffer preparation

The amount of buffer was added according to the volume of plasma to maintain ratio. For this, 0.500 mL of 10 mmol/L Ammonium Formate pH 4.00 ± 0.05 buffer per 1 mL of plasma was added. Plasma samples were placed in biological sample storage box and the samples were then placed in deep freezer maintained at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The samples were further estimated for itraconazole and hydroxy-itraconazole by a validated LC-MS/MS analytical method.

Pharmacokinetic- pharmacodynamics analysis

PK parameters for each subject-formulation combination were calculated by non-compartmental model using SAS® version 9.4. The following PK parameters were calculated: Primary PK parameters included; AUC_{0-t} - AUC - time curve measured to the last sampling time (AUC_{0-t}), using the linear trapezoidal rule, and $\text{AUC}_{0-\infty}$ - AUC_{0-t} + additional area extrapolated to infinity, calculated as $\text{AUC}_{0-t} + C_t/\text{Kel}$, where C_t is last measurable drug concentration and Kel is the elimination rate constant.

PK parameters AUC_{0-t} and $\text{AUC}_{0-\infty}$ were taken as primary efficacy variables for assessing bioequivalence between the test product (T) and reference product (R2).

Secondary PK parameters included: C_{max} - maximum drug concentration (ng/mL); T_{max} - time to reach maximum drug concentration; $\text{AUC}_{0-t}/\text{AUC}_{0-\infty}$ - ratio of AUC_{0-t} and $\text{AUC}_{0-\infty}$; Kel - First-order elimination rate constant calculated from a semi-log plot of the plasma concentration *vs* time curve, using the method of least square regression, $t_{1/2}$ - first-order terminal elimination half-life, residual area - extrapolated area ($\text{AUC}_{0-\infty} - \text{AUC}_{0-t}$)/ $\text{AUC}_{0-\infty}$; PD parameter AUC/MIC - area under plasma concentration/ minimum inhibitory concentration.

Statistical analysis

All statistical analyses were performed using SAS® version 9.4. The plasma concentrations at each sampling time point were tabulated for each subject and product combination. Descriptive statistics for each product at each scheduled sampling time point was done.

All concentration values below the limit of quantification (BLQ) were to be set to zero for the estimation of pharmacokinetic parameters.

ANOVA on the log transformed PK parameters C_{max} , AUC_{0-t} and $\text{AUC}_{0-\infty}$ at α level of 0.05 for itraconazole and Hydroxy itraconazole using Proc MIXED model.

Test product was concluded bioequivalent to the reference product (R2) if 90% confidence interval (CI) of geometric mean ratio of log transformed pharmacokinetic parameters AUC_{0-t} and $\text{AUC}_{0-\infty}$ between test (T) and reference (R2) products fall within the range of 80.00% to 125.00% for itraconazole. Additionally, supportive data on active metabolite hydroxy-itraconazole was analyzed. For each subject, AUC/MIC ratio was calculated for test as well as reference products considering three different levels of MIC, *i.e.*, 4, 8 and 16 mcg/mL. For each MIC level, $\text{AUC}/\text{MIC} > 25-50$ was considered for comparison between test and reference (R1) treatment. Mean AUC/MIC is considered > 50 for optimal efficacy at these dose levels. For each MIC levels, data on the number of subjects having $\text{AUC}/\text{MIC} > 25-50$ and $\text{AUC}/\text{MIC} > 50$ was analyzed for comparison between test and reference (R1) treatments using two independent sample T Test statistical test (binomial test).

Safety assessment

Safety parameters included, physical examination assessed at the time of screening, during study and post study. Vital signs (blood pressure, pulse rate), wellbeing assessment and 12 Lead ECG was done at the time of screening, pre and post dose at 01.00, 03.00, 05.00 and 08.00 h, in each study period, during check out and post study. Haemogram, urinalysis and biochemistry profile (lipid profile), serum electrolytes test was done during screening and post study. Adverse events (AEs) assessments were performed in each study period and post study evaluation.

RESULTS

Demographic characteristics and sample size determination

A total of 54 subjects were enrolled in the study, comprising of healthy adult males ($n = 44$) and females ($n = 10$). Three subjects were withdrawn due to AEs; one subject had fever in period III during 36 h post-dose ambulatory visit, another subject had loose motion in period I pre-dose and the third subject was withdrawn from the study period I post-dose due to vomiting. Data of the subjects completing at least 2 periods of the study (provided subject has received the test product in any one of the 2 periods attended) were considered for PK and statistical analysis. Considering the estimate of T/R ratio 95 to

105 %, intra-Subject C.V (%) 34% and significance level 5%, power $\geq$ 80% and bioequivalence Limits 80.00 – 125.00% (C_{max} and AUC), a sample size of 50 subjects was sufficient to establish bioequivalence between formulations with adequate power. However, considering the drop-out or withdrawn, a sample size of 54 ± 2 subjects was considered for the statistical analysis of test *vs* reference product (R2).

The mean $\pm$ standard deviation (SD) range of age, and BMI of 52 participants were: 30.74 ± 5.52 years (20-42 years) and 24.68 ± 3.40 kg/m² ($18.99 - 29.80$ kg/m²), respectively.

Pharmacokinetics

Primary PK variables assessed for bioequivalence of test and reference products (R2) for itraconazole were compared statistically. The 90% CI value for PK parameters AUC_{0-t} and AUC_{0-inf} for itraconazole were 90.66 % (83.31% - 98.66%) and 88.92 % (81.58%, 96.93%), respectively. Values for both the parameters were well within the equivalence range of 80 to 125% for itraconazole. Data presented in Table 1 determine that both the test and reference product (R2) were bioequivalent under fed conditions.

The mean C_{max} values for itraconazole after administration of test and reference product (R2) were 134.47ng/mL and 136.69 ng/mL respectively. Time taken to reach the maximum concentration level in plasma was 10.0 (4.5 to 24.12) h for the test product, and 8.5 (4.5 to 24.02) h for reference product (R2). The mean AUC ratio for the test and reference product (R2) was recorded as 88.04% and 87.63%, respectively.

For PD parameter AUC/MIC, the test product (T) was considered as comparable with the reference product for each MIC level if there exists no statistically significant difference ($P > 0.05$ and observed $P = 0.3196$).

ANOVA analysis

In analysis of variance model of itraconazole, no significant sequence and period effect were observed for C_{max} ($P = 0.6848, 0.6595$), AUC_{0-t} ($P = 0.5778, 0.1998$) and AUC_{0-inf} ($P = 0.6430, 0.1660$). In addition, no significant 'treatment' effect was observed for C_{max} and AUC_{0-t} ($P = 0.8548$ and 0.0575). However, significant 'treatment' effect was observed for AUC_{0-inf} ($P = 0.0268$).

Safety analysis

During the course of study, a total of six AEs were reported. All the AEs were mild to moderate in severity. Four (7.40%) AEs were reported by two participants in the test group (feeling feverish and fever), while one (1.85%) AE was reported by a subject in the reference (R1) group (vomiting) and the other AE (1.85%) was observed in a subject in the reference (R2) group (headache). All the AEs were resolved. No severe AE (SAEs) was observed during the study.

DISCUSSION

Bioequivalence studies form an integral core in the new drug development process[18]. According to United States Food and Drug Administration (FDA), two pharmaceutically equivalent products are considered bioequivalent if the 90% CI of geometric mean ratio (GMR) of primary PK (C_{max} , AUC_{0-t} and $AUC_{0-\infty}$) variables fall within the range of 80%-125%[19]. As per the European Medicines Agency (EMA) guidelines, a randomized, two period, two sequence, single dose, crossover design must be used for the comparison of two drug formulations. The current clinical trial was conducted in accordance with FDA guidance and EMA recommendations[19,20].

Although this was an open label study, the analyst was blinded to the sequence of test and reference products (R1 and R2) administered to the subjects. EMA recommends a washout interval after each period to ensure that drug concentrations are below the lower limit among participants, before the next period commences[20]. Similarly, in our study to avoid any residual or carry over effect, a washout period of 14 days between period I and period II and 20 days between period II and period III was given. In addition, no significant period effect was observed for C_{max} , AUC_{0-t} and AUC_{0-inf} for itraconazole.

This study was performed under fed conditions as the absorption of itraconazole capsule is facilitated in acidic environment[4,21]. It is also stated that the oral bioavailability of itraconazole gets doubled when administered after food[10,22]. Conversely, absorption is impaired with concomitant use of antacids, or proton pump inhibitors. However, no food effect is observed in case of oral suspension with oral bioavailability is enhanced in fasting state[4,23].

In the current study, statistical analysis of primary PK variables AUC_{0-t} and AUC_{0-inf} revealed bioequivalence, with CIs being completely inside the acceptance criteria of 80-125% as suggested by FDA and EMA[19,20]. The statistical power was greater than 80% for itraconazole.

Following administration of test and reference product (R2), the peak concentration levels of itraconazole were achieved at 10 h and 8.5 h, respectively. Other parameters including C_{max} , K_{el} , and $t_{1/2}$ for test and reference product (R2) compared well. The mean pharmacokinetic parameters for test and Reference Product (R1 and R2) for itraconazole are summarized in Table 2. Hydroxyitraconazole data

Table 1 Summary of pharmacokinetic variables determining bioequivalence for itraconazole – Test vs reference product (R2)

Variable	Geometric mean		T/R2 ratio (%), 90%CI	Outcome of BE result
	Test (T)	Reference (R2)		
AUC _{0-t} (ng × h/mL)	2533.47	2794.45	0.9066 (83.31% - 98.65%)	Bioequivalent
AUC _{0-inf} (ng × h/mL)	2856.78	3212.68	0.8892 (81.57% - 96.92%)	Bioequivalent
C _{max} (ng/mL)	117.4	118.82	0.988 (88.57%-110.22%)	Bioequivalent

T: Test; R2: Reference; BE: Bioequivalence; CI: Confidence interval; AUC_{0-t}: Area under the concentration-time curve over the dosing interval; AUC_{0-inf}: Area under the curve; C_{max}: Maximum concentration; T_{max}: Time to peak drug concentration.

Table 2 Mean pharmacokinetic parameters for test and reference product (R1 and R2) for itraconazole

Variable	mean ± SD		
	Test (T)	Reference (R1)	Reference (R2)
C _{max} (ng/mL)	132.12 (65.31)	308.57 (173.63)	136.90 (67.91)
AUC _{0-t}	2738.083 (1164.121)	4953.205 (2472.121)	3065.766 (1295.989)
AUC _{0-inf}	3109.578 (1342.394)	5744.760 (2917.508)	3614.170 (1850.356)
T _{max}	11.45(6.05)	6.00 (1.49)	10.85 (6.33)
Kel	0.022 (0.007)	0.021 (0.007)	0.023 (0.008)
t _{1/2}	34.89 (13.18)	37.83 (14.95)	35.16 (20.14)
Residual area	11.92 (7.41)	12.86 (7.86)	12.22 (9.10)

SD: Standard deviation; AUC_{0-t}: Area under the concentration-time curve over the dosing interval; AUC_{0-inf}: Area under the curve; C_{max}: maximum concentration; T_{max}: Time to peak drug concentration.

Table 3 Mean pharmacokinetic parameters for test and reference product (R1 and R2) for hydroxyitraconazole

Variable	mean ± SD		
	Test	Reference (R1)	Reference (R2)
C _{max} (ng/mL)	181.35 (58.30)	310.20 (11.63)	197.12 (56.84)
T _{max}	12	8	12
Kel	0.050 (0.015)	0.043 (0.014)	0.050 (0.019)
t _{1/2}	15.20 (4.92)	18.14 (7.10)	16.01 (6.55)
Residual area	2.72 (3.12)	4.31 (5.03)	3.37 (3.88)

SD: Standard deviation; T_{max}: Time to peak drug concentration; C_{max}: maximum concentration.

was analyzed statistically and used only as supportive data. The mean pharmacokinetic parameters for test and Reference Product (R1 and R2) for Hydroxyitraconazole are summarized in Table 3.

AUC/MIC correlates with efficacy of anti-fungal drug and its clinical response, *i.e.*, microbiological cure and clinical outcome of antifungal agents. Pharmacodynamic parameter assessment showed that AUC/MIC for conventional itraconazole 200 mg are comparable to the novel improved bioavailable itraconazole 100 mg for MIC levels upto 16 µg/mL. This indicates that similar therapeutic effect is expected with half dose of supra bioavailable itraconazole as compared to conventional itraconazole [15].

Supra bioavailable itraconazole capsules (Fixtral SB) were found to be safe and well tolerated which is in line with historical data.

CONCLUSION

The test product, itraconazole capsule 100 mg is bioequivalent with the reference product (R2) at 100 mg dose (2 capsules of Lozanoc®50 mg) under fed conditions. The pharmacodynamics activity in terms of AUC/MIC is comparable between test product at 100 mg dose and marketed itraconazole 200 mg. Based on the results obtained, Fixtral SB is expected to have therapeutically similar efficacy at half the equivalent dose. All the tested formulations were found to be safe and well tolerated with resolvable side-effects.

ARTICLE HIGHLIGHTS

Research background

Itraconazole is a broad-spectrum triazole antifungal. It inhibits fungal growth. It exhibits a nonlinear pharmacokinetic profile due to its varied oral bioavailability.

Research motivation

The test product Fixtral SB is a new formulation that has been manufactured *via* APSG™ (Advanced Polymeric Spray Granulation), a breakthrough innovative proprietary technology for enhancing solubility and maximizing the bioavailability of poorly soluble drug itraconazole. The new formulation is able to restrict the drug release in an acidic environment which minimizes the precipitation potential of itraconazole in the intestine thus maximizing bioavailability.

Research objectives

To compare the oral bioavailability and bioequivalence of Fixtral supra bioavailable itraconazole (SB) with reference product R2 (supra bioavailable 50 mg itraconazole × tablets).

Research methods

The comparison of Fixtral SB (supra bioavailable itraconazole) 100 mg with Lozanoc capsules 50 mg administered as two capsules was performed, revealing that Fixtral SB is expected to have therapeutically comparable efficacy at half the equivalent dose. In terms of area under the concentration-time curve (AUC) / minimum inhibitory concentration (MIC), the pharmacodynamic activity of the test product at 100 mg dose and the marketed itraconazole 200 mg is comparable. All of the formulations tested were found to be safe and well tolerated, with manageable side effects.

Research results

Based on the results, Fixtral SB is expected to have therapeutically similar efficacy at half-equivalent doses. All the tested formulations were found to be safe and well tolerated with resolvable side effects.

Research conclusions

The peak concentration levels of itraconazole were achieved at 10 h (T) and 8.5 h (R2), respectively. Pharmacodynamic parameter assessment shows that AUC/MIC for R1 is comparable to Fixtral SB 100 mg for MIC levels up to 16 µg / mL.

Research perspectives

The study population consisted of 54 healthy male and female volunteers, aged between 18-45 years. Subjects were randomized to receive a single oral dose of either test product (T) or reference product (R1 and R2). Blood samples were taken pre-dose and post-dose for up to 96 h to evaluate the bioequivalence. Adverse events assessments were performed in each study period and post-study evaluation.

FOOTNOTES

Author contributions: Syed N, Monil G, Snehal M, Anand A, Dhananjay P, Dipak P, Rahul R, Amey M equally contributed to this work; designed research; performed research; analyzed data and drafted the paper; all authors provided critical feedback and helped shape the research, analysis, and manuscript.

Institutional review board statement: The study protocol, informed consent forms and other relevant documents (subjects' accrual) were reviewed and approved by 'Ethicare Ethics committee'. Pre-screening and general safety instructions in view of COVID-19 pandemic were reviewed and approved by 'Human Care Independent Ethics Committee'. The study was performed in compliance with the principles of the Declaration of Helsinki for Medical Research involving human subjects, the Guideline for Good Clinical Practice and Drug Control General of India regulatory guidelines.

Clinical trial registration statement: This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki [Ethical Principles for Medical Research Involving Human Subjects, Revised by 71th WMA General Assembly at Cordoba, Spain (virtual), in 2020] and that were consistent with Good Clinical Practices and Drug Control General of India regulatory guidelines.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors report that they are employees of Dr Reddy's Laboratories Ltd. The authors report no other conflicts of interest in this work.

Data sharing statement: The datasets are available only on request due to privacy/ethical restrictions.

CONSORT 2010 statement: The authors have read and filled out the CONSORT Checklist.

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Country/Territory of origin: India

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S-Editor: Ma YJ

L-Editor: A

P-Editor: Ma YJ

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World Journal of *Pharmacology*

World J Pharmacol 2023 March 22; 12(2): 12-17



CASE REPORT

- 12 Recurrent ciprofloxacin induced hypoglycemia in a non-diabetic patient: A case report

Dasuqi SA, Alshaer LM, Omran RA, Hamad MA

ABOUT COVER

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INDEXING/ABSTRACTING

The WJP is now abstracted and indexed in Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Xiang-Di Zhang; Production Department Director: Xiang Li; Editorial Office Director: Yun-Xiaojiao Wu.

NAME OF JOURNAL

World Journal of Pharmacology

ISSN

ISSN 2220-3192 (online)

LAUNCH DATE

February 9, 2012

FREQUENCY

Continuous Publication

EDITORS-IN-CHIEF

Pharkphoom Panichayupakaranant, Ahmed M Kabel, Muhammad Shahzad

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2220-3192/editorialboard.htm>

PUBLICATION DATE

March 22, 2023

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<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

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<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

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Recurrent ciprofloxacin induced hypoglycemia in a non-diabetic patient: A case report

Shereen A Dasuqi, Linah M Alshaer, Rasha A Omran, Mohammed A Hamad

Specialty type: Endocrinology and metabolism

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Su G, China

Received: November 25, 2022

Peer-review started: November 25, 2022

First decision: January 31, 2023

Revised: February 13, 2023

Accepted: March 9, 2023

Article in press: March 9, 2023

Published online: March 22, 2023



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Abstract

BACKGROUND

Fluoroquinolones are a class of broad-spectrum antimicrobials used for various bacterial infections. Frequent use of fluoroquinolones has been questioned due to severe associated adverse effects, including dysglycemia (hypoglycemia or hyperglycemia) due to an alternation in glucose metabolism. Recent clinical trials showed the association of poor clinical outcomes with hypoglycemia in critically ill patients without diabetes. Many predisposing factors worsen fluoroquinolone-induced hypoglycemia, including diabetes, concomitant medication use like sulfonyleureas or insulin, renal disease, and the elderly.

CASE SUMMARY

We report a case of recurrent hypoglycemia after ciprofloxacin initiation for a 71-year-old, non-diabetic, critically ill patient despite the presence of total parenteral nutrition and nasogastric tube feeding. The adverse drug reaction probability (Naranjo) scale was completed with a probable adverse drug reaction. The hypoglycemia resolved entirely after ciprofloxacin discontinuation.

CONCLUSION

Although ciprofloxacin-induced hypoglycemia is rare, special consideration is

needed for the elderly due to their higher susceptibility to adverse side effects.

Key Words: Ciprofloxacin; Hypoglycemia; Fluoroquinolones; Total parenteral nutrition; Side effect; Case report

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Core Tip: Fluoroquinolones are a class of broad-spectrum antimicrobials used to treat various bacterial infections. Frequent use has been doubted due to associated adverse severe effects, including hypoglycemia or hyperglycemia due to an alternation in glucose metabolism. Recent clinical trials showed the association of poor clinical outcomes with hypoglycemia in critically ill patients without diabetes, emphasizing the harm of severe hypoglycemia. Ciprofloxacin has demonstrated the lowest incidence in the class in the diabetic population. We report a case of recurrent ciprofloxacin-induced hypoglycemia for non-diabetic critically ill patient despite total parenteral nutrition and nasogastric tube feeding. The hypoglycemia resolved successfully after ciprofloxacin discontinuation.

Citation: Dasuqi SA, Alshaer LM, Omran RA, Hamad MA. Recurrent ciprofloxacin induced hypoglycemia in a non-diabetic patient: A case report. *World J Pharmacol* 2023; 12(2): 12-17

URL: <https://www.wjgnet.com/2220-3192/full/v12/i2/12.htm>

DOI: <https://dx.doi.org/10.5497/wjp.v12.i2.12>

INTRODUCTION

Fluoroquinolones are broad-spectrum antimicrobials used for different bacterial infections, especially for treating community-acquired pneumonia and intraabdominal and urinary tract infections. Fluoroquinolones exhibit bacteriostatic activity by inhibiting deoxyribonucleic acid (DNA) gyrase, the enzyme responsible for replicating DNA, while their bactericidal activity is achieved by bacterial DNA fragmentation[1].

The frequent use of fluoroquinolones has been questioned due to rare but severe associated adverse effects such as heart valve regurgitation, dysglycemia (hypoglycemia or hyperglycemia) due to alternations in glucose metabolism, and irreversible side effects to the musculoskeletal and nervous systems - most commonly tendonitis and tendon ruptures that can lead to potentially long-lasting disability[2-4]. Adverse drug events (ADEs) can be life-threatening, especially in the critically ill population. Many factors like patient characteristics, environmental factors, and drug classes related to ADEs affect intensive care differently than general care units. Hypoglycemia was associated with increased intensive care unit (ICU) length of stay and increased utilization of mechanical ventilation, catecholamines, and renal replacement therapy[5].

Many predisposing factors are known to increase the incidence of hypoglycemia in patients treated with fluoroquinolones, including diabetes, concomitant use of some medication like sulfonylureas or insulin, renal insufficiency, and elderly (conventionally defined as greater than 65 years old)[6]. We report a case of recurrent hypoglycemia in a non-diabetic patient that resolved successfully after ciprofloxacin discontinuation.

CASE PRESENTATION

Chief complaints

Unexplained recurrent hypoglycemia.

History of present illness

On the first day following abdominal closure, enteral feeding was initiated through a nasogastric tube (NGT), and the following day total parenteral nutrition (TPN) was initiated to augment his nutritional state. As a result, his blood glucose readings rose and were maintained above 99 mg/dL for the rest of his stay. Furthermore, as the patient tolerated the NGT feeding well, it was gradually increased over the following days.

Due to worsening sepsis and increased vasopressor requirements, 400 mg of ciprofloxacin was administered intravenously (IV) every 12 h and adjusted accordingly on day two following surgery, based on a positive culture result. Approximately 30 h later, the patient had an episode of symptomatic hypoglycemia and received 12.5 mg of glucose IV, which somewhat improved his blood glucose level.

However, his relatively low blood glucose readings continued despite increasing calories through TPN and NGT feeding. Therefore, Additional dextrose infusion was added to avoid further hypoglycemic episodes. After reviewing all the medications and evaluating his caloric intake during the daily rounds, we suspected that the ciprofloxacin might be the culprit behind the patient's hypoglycemia. Retrospectively, we examined his most recent blood glucose readings and observed a downward trend in his blood glucose readings that started immediately following the second dose of ciprofloxacin refer to [Figure 1](#). Accordingly, it was discontinued and the antimicrobial strategy was modified.

History of past illness

Our patient was a 71-year-old male who suffered from hypertension, peripheral vascular disease, and dyslipidemia, with a previous history of aortobifemoral bypass surgery. He was admitted to the hospital following a two-month complaint of abdominal pain. On admission his pain was moderate, and he was hemodynamically stable with a blood glucose concentration of 126 mg/dL, Glasgow Coma Scale/Score of 15/15, and unremarkable lab results.

He underwent exploratory laparotomy due to possible bowel ischemia, but only dusky bowel was found with no evidence of perforation or ischemia. His hospital course was complicated, requiring multiple ICU admissions for various reasons including sepsis, septic shock, acute kidney injury (AKI), abdominal fluid collection, and electrolyte imbalances.

One month after his hospital admission, he underwent another exploratory laparotomy due to wound dehiscence and a bowel perforation. Multiple micro-perforations in the small bowel and bowel ischemia were discovered during surgery, the abdomen was kept open, and he required three more surgeries afterward. His final fifth surgery was for abdominal closure.

Personal and family history

Not relevant to the case.

Physical examination

Not relevant to the case.

Laboratory examinations

Multiple glucose blood levels were recorded in correlation to ciprofloxacin administration.

Imaging examinations

Not relevant to the case.

FINAL DIAGNOSIS

Ciprofloxacin induced hypoglycemia.

TREATMENT

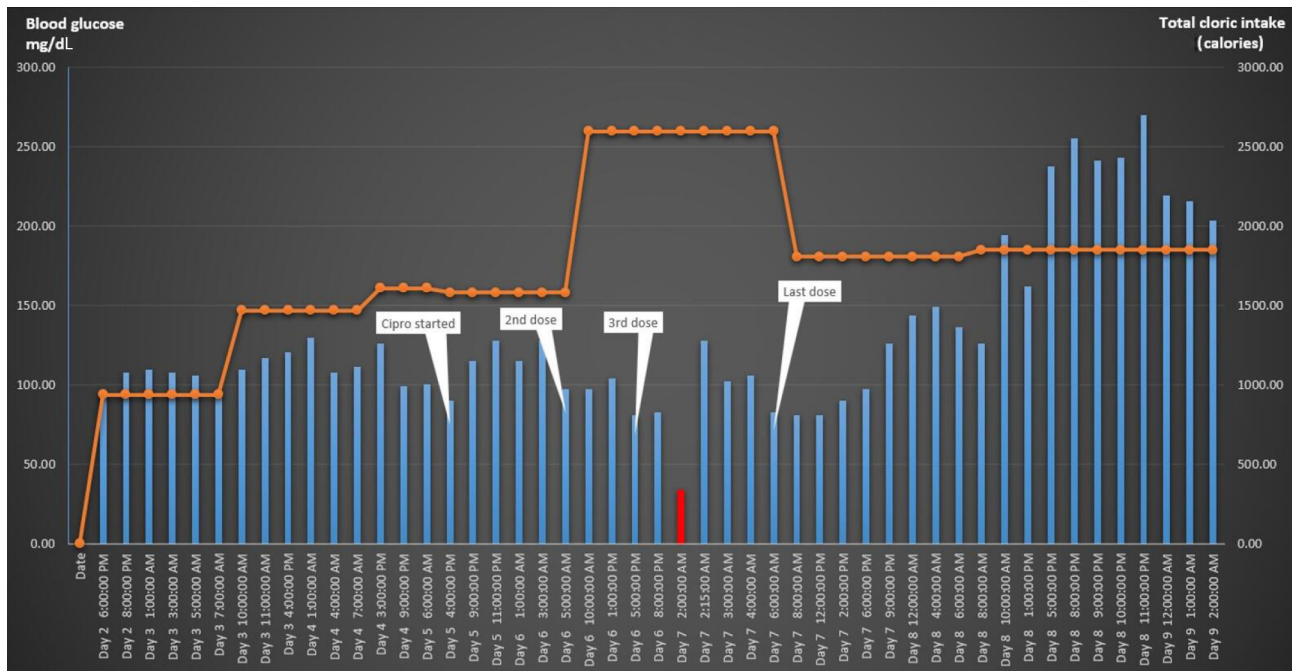
Increasing calories through TPN and NGT feeding. Additional dextrose infusion was added as needed to maintain normal blood sugar. After ruling out other possible causes, ciprofloxacin was discontinued.

OUTCOME AND FOLLOW-UP

After 12 h of ciprofloxacin discontinuation, all his readings were 126 mg/dL, and the dextrose infusion was stopped. 24 h later, and despite decreasing the enteral feeding amount due to a high gastric residual resulting in a significant decrease in his total caloric intake, insulin infusion had to be initiated to control his blood glucose readings refer to [Figure 1](#).

DISCUSSION

Recent clinical trials have shown a connection between poor clinical outcomes and hypoglycemia in critically ill patients without diabetes, emphasizing the harm caused by severe hypoglycemia and challenging the intensive glycemic control strategy to manage both diabetic and severe ill patients. Although hypoglycemia is unusual in patients without diabetes, many etiologies can cause hypoglycemia, including sepsis, liver diseases, malnutrition, alcohol-related illnesses, malignancies, post gastrectomy syndrome, and endocrine disorders[7].



DOI: 10.5497/wjp.v12.i2.12 Copyright ©The Author(s) 2023.

Figure 1 Details the relationship between the administration of ciprofloxacin and the patient's blood sugar levels and total daily caloric intake.

Fluoroquinolones are antibiotics widely used to treat various bacterial infections, but several adverse effects have been reported with their use. In 2018, The US Food and Drug Administration (FDA) announced new drug safety communications regarding fluoroquinolones and the risk of hypoglycemia. Their reports showed that levofloxacin caused most of the incidents, followed by ciprofloxacin. In the end, the FDA strengthened the warnings to the prescriber that fluoroquinolone antibiotics may cause significant reductions in blood sugar and specific mental health side effects[8,9]. Many retrospective studies have shown that fluoroquinolones significantly interfere with glucose homeostasis and insulin secretion, leading to hypoglycemia and hyperglycemia events. The pathophysiological mechanism of hypoglycemia associated with fluoroquinolones is not yet fully understood. However, animal and *in vitro* studies hypothesized that fluoroquinolones block adenosine triphosphate-sensitive potassium channels in pancreatic β -cells, which in turn leads to an increase in insulin release[10-13].

Two large retrospective cohort studies by Mohr *et al*[14] and Aspinall *et al*[15] on diabetic outpatients revealed the high risks of hypoglycemia associated with fluoroquinolone treatment. However, the risk of a clinically-relevant dysglycemic event appears to vary highly among this class of drugs, with significantly greater risks for gatifloxacin and levofloxacin compared to ciprofloxacin. Chou *et al*[3] conducted a retrospective cohort study among 78433 outpatient diabetic patients for 22 mo; the study results identified an association between fluoroquinolones and a higher risk of hypoglycemia. Moxifloxacin was associated with the highest risk of hypoglycemia, followed by levofloxacin, with ciprofloxacin carrying the least risk[16].

Many case reports have documented levofloxacin-induced hypoglycemia in diabetic patients, and one case report detailed fatal hypoglycemia with ciprofloxacin in a diabetic patient with end-stage renal disease due to diabetic neuropathy[13,17-19].

A 2019 research article suggested an association between ciprofloxacin and hypoglycemia in patients without diabetes. They searched the World Health Organization's global adverse drug reaction database using "ciprofloxacin" as the medicine name and "hypoglycemia" as the reaction term. A total of 35 cases from 17 countries were found since 1989 for patients without diabetes developed with ciprofloxacin-induced hypoglycemia, with a median time-to-onset of four days. The cases had a median age of 64 years (interquartile range, 50-85) with a balanced male-to-female ratio (but one of the limitations was that hyperinsulinemia or diabetes could not be ruled out)[17].

Our patient's Naranjo Adverse Drug Reaction Probability Scale was six, which indicates a "probable adverse drug reaction". However, the Naranjo score may have limitations in evaluating adverse drug reactions in critically ill patients, such as difficulty re-challenging patients, the inapplicability of placebo administration, possible lack of serum drug concentrations, and clarification of objective measurement.

Hyperglycemia is a known metabolic complication of TPN, due to the presence of dextrose as the primary energy source in TPN, that commonly occurs in diabetes mellitus or insulin-resistant patients. It can occur in patients without diabetes as well, with risk increasing due to the presence of other risk factors such as age, illness severity, and TPN infusion rate. Hypoglycemia, by contrast typically occurs

in cases of excess insulin administration *via* parenteral nutrition solution. Usually, the risk of hypoglycemia increases along with certain patient characteristics (e.g. age), medications, and comorbidities (e.g. diabetes mellitus, mechanical ventilation, renal frailer, sepsis, and nutritional status) [12].

In our case, the patient was euglycemic from his initial hospital admission, despite developing severe sepsis and septic shock several times before that latest encounter without any episodes of hypoglycemia that eliminated the severe illness condition as the sole reason for hypoglycemia. Moreover, during the incident mentioned above, and in response to his low blood glucose readings, our medical team, nutritionist, and clinical pharmacist tried to address the issue by increasing the patient's total caloric intake through various sources, including starting dextrose infusions, maximizing his TPN dose, and diluting all his IV medications in dextrose solutions. The risk of mechanical ventilation and AKI-induced hypoglycemia, as well as other possible comorbidities, are negligible for this case because these comorbidities did not induce hypoglycemic events before initiating ciprofloxacin. The only risk factor that may have predisposed our patient to hypoglycemia while receiving fluoroquinolone was his advanced age of 71. Renal function decreases as part of the usual aging pathophysiology, even without concurrent renal disease. That results in the accumulation of drugs that depends on renal excretion as the primary eliminated route, like ciprofloxacin. Impaired drug clearances affect the kinetics of drugs in prolonged half-lives, high drug serum concentration and increased side effects and toxicity[20].

Interestingly, after stopping ciprofloxacin, he started to become hyperglycemic, and although his caloric intake was reduced as a response, he required insulin infusion to achieve proper glycemic control.

The prevalence of ADEs increases with age due to age-related changes in pharmacokinetics and pharmacodynamics, increasing comorbidity burden, and polypharmacy. This makes older patients more sensitive to the side-effects of medications than their younger counterparts. A trained clinician can predict and detect ADEs in vulnerable patients by maintaining detailed documentation and regularly reviewing all prescribed and over-the-counter medications through standardized medication reconciliation. It is also important to outline clear therapeutic goals and recognize various drugs' impacts on multiple organ systems for newly prescribed medications[15].

CONCLUSION

Although ciprofloxacin is rare to cause hypoglycemia, it can lead to fatal complications. Therefore, it should be prescribed carefully, after considering the patient's risk factors, especially the geriatric population who are more susceptible to adverse side-effects.

FOOTNOTES

Author contributions: All the authors wrote the manuscript, contributed to the case report, interpreted the data, and edited the manuscript; All authors have read and agreed to the published version of the manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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S-Editor: Liu JH

L-Editor: A

P-Editor: Liu JH

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World Journal of *Pharmacology*

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INDEXING/ABSTRACTING

The WJP is now abstracted and indexed in Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Mai-Xin Ji; Production Department Director: Xiang Li; Editorial Office Director: Yun-Xiaojiao Wu.

NAME OF JOURNAL

World Journal of Pharmacology

ISSN

ISSN 2220-3192 (online)

LAUNCH DATE

February 9, 2012

FREQUENCY

Continuous Publication

EDITORS-IN-CHIEF

Pharkphoom Panichayupakaranant, Ahmed M Kabel, Muhammad Shahzad

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2220-3192/editorialboard.htm>

PUBLICATION DATE

June 16, 2023

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INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

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PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

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Commentary: Discussing the antidepressant potential of silymarin

Claire E Manhard, Brandon Lucke-Wold

Specialty type: Pharmacology and pharmacy

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Liu XQ, China

Received: January 20, 2023

Peer-review started: January 20, 2023

First decision: April 28, 2023

Revised: May 10, 2023

Accepted: May 31, 2023

Article in press: May 31, 2023

Published online: June 16, 2023



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Abstract

The therapeutic potential of diet, dietary supplements, herbal remedies, and nutraceuticals for treatment of depression and anxiety is being increasingly explored. In this commentary, we discuss the recent findings on the antidepressant potential of silymarin (SILY) in mice and present an alternative approach. We highlight the extensive research on another phytochemical, curcumin, for the treatment of depression and anxiety. Finally, we suggest a future application, which investigates the potential synergistic effects of combined treatment with SILY and curcumin for depression.

Key Words: Silymarin; Depression; Anxiety; Phytochemicals; Nutraceuticals; Curcumin

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Core Tip: There are several reviews focused on the role of silymarin (SILY) in chronic diseases, however, there is a paucity of literature reviewing the potential antidepressant effects of SILY. This commentary serves as a discussion of the recent findings regarding the antioxidant, anti-inflammatory, and antidepressant-like potential of SILY in mice and as a catalyst for future discovery in phytochemistry.

Citation: Manhard CE, Lucke-Wold B. Commentary: Discussing the antidepressant potential of silymarin. *World J Pharmacol* 2023; 12(3): 18-24

URL: <https://www.wjgnet.com/2220-3192/full/v12/i3/18.htm>

DOI: <https://dx.doi.org/10.5497/wjp.v12.i3.18>

INTRODUCTION

The paper titled "Antidepressant-like potential of silymarin and silymarin-sertraline combination in mice: Highlighting effects on behavior, oxidative stress, and neuroin-

flammation” by Onaolapo *et al*[1] demonstrates the ability of silymarin (SILY) alone or in conjunction with oral sertraline to regulate behavior, oxidative stress, and neuroinflammation in dexamethasone (DEX)-induced depression in mice[2]. A summarization of this study’s main findings regarding the effects of SILY alone can be found in Figure 1.

SILY, which is an active compound from the *Silybum marianum* L. Gaernt. herb, commonly called milk thistle, has antifibrotic, anti-inflammatory, and antioxidant properties[2-4]. Due to SILY’s ability to prevent free radical formation and modify enzymes, its hepatoprotective effects have been well studied [5,6]. More recently, research has explored SILY’s potential as a treatment for alcoholic liver cirrhosis[7]. Emerging evidence has suggested SILY’s anti-viral[8], anti-cancer[9], anti-Alzheimer’s disease[10], anti-Parkinson’s disease[11], and anti-diabetic[12] therapeutic effects.

Depression, a complex neuropsychiatric disorder, is a leading cause of disability, a large contributor to global disease burden, and is associated with high suicidality[13]. One longstanding hypothesis for the development of depression is the “serotonin hypothesis”[14]. This hypothesis posits that serotonin (5-hydroxytryptamine) plays a critical role in the pathophysiology of depression[15]. Conventional treatment of depression with serotonin reuptake inhibitors (SSRIs) acts by blocking the reuptake of serotonin on the presynaptic neuron, thus increasing the amount of serotonin in the synaptic cleft and postsynaptic neuronal activity[16].

As the precise etiology of depression remains unknown, researchers have begun to investigate the potential benefits of mechanisms of action that go beyond neurotransmitter modulation such as the use of dietary supplements and herbal remedies[17,18]. Further, there is increasing evidence of the role of inflammation and oxidative stress in the pathogenesis of depression[19]. Several studies have demonstrated activated inflammatory pathways in patients with depression with increased acetylcholinesterase activity, malondialdehyde (MDA), tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1, IL-2, and IL-6, and decreased IL-10[20-26]. Interestingly, these inflammatory markers may also directly impact the release of serotonin and/or the production of serotonin[27,28]. Radical-induced oxidative stress has also been shown to be linked to depression, with increased superoxide dismutase (SOD) and decreased glutathione (GSH) peroxidase (GPx), GSH, and catalase in both nonclinical and clinical studies[20,29,30].

DISCUSSION OF ONAOLAPO ET AL

Given the association of inflammation and oxidative stress with depression, Onaolapo *et al*[1] sought to elucidate the potential antidepressant effect of SILY extract alone or in conjunction with a well-known SSRI, sertraline in an animal model. DEX, which was used as the mice model of depression, has been demonstrated to be effective at creating a low levels of corticosteroids in the brain and inducing depression and anxiety-like behaviors[31,32]. A total of 10 treatment groups were included: 1 vehicle control group, 1 DEX group, 1 oral sertraline group, 1 SILY 140 mg/kg group and 1 SILY 280 mg/kg group, 1 DEX and sertraline group, 1 DEX and SILY 140 mg/kg group, 1 DEX and SILY 280 mg/kg group, 1 DEX, sertraline, and SILY 140 mg/kg group, and 1 DEX, sertraline, and SILY 280 mg/kg group.

SILY’s effects on body weight, activity, and memory

Onaolapo *et al*[1] found that SILY administered alone with DEX or with sertraline and DEX reversed the DEX-induced changes in mouse body weight, open field locomotor activity, rearing, and grooming. Body weight increased when mice were given SILY alone as compared to the control or sertraline. While SILY affected body weight, there was no significant change in food intake with SILY alone when compared to control, sertraline, or DEX. Interestingly, Guo *et al*[10] found that SILY administration in mice that were fed a high fat diet resulted in a decrease in body weight and a clinical trial by Momeni *et al*[33] revealed that treatment with SILY following cisplatin was able to significantly increase weight back to baseline. Onaolapo *et al*[1] findings in conjunction with results from Guo *et al*[10] and Momeni *et al*[33] suggest that SILY modulates body weight in a way that returns body weight back to a normal value.

Additionally, there was also a SILY concentration dependent increase in locomotor activity. Rearing activity in the SILY group was not significantly different from control; however, low dose SILY rearing activity was significantly greater than sertraline. With regards to self-grooming behavior, only the low dose of SILY resulted in a significant increase in self-grooming behaviors in comparison to control and sertraline. While there was a relatively unclear dose relationship between SILY alone and grooming behaviors, it is important to note that there was a significant attenuation of DEX-induced decreases when mice were administered SILY at both doses with DEX or SILY at both doses with sertraline and DEX. This corroborates Ashraf *et al*[34] findings that SILY administration at 200 mg/kg in chronically stressed mice resulted in a significant increase in grooming time.

Onaolapo *et al*[1] reported that spatial working memory, tested using the radial arm maze and Y maze, was increased in mice administered SILY. SILY administered alone at both concentrations increased working memory scores as compared to the vehicle control and sertraline, and SILY

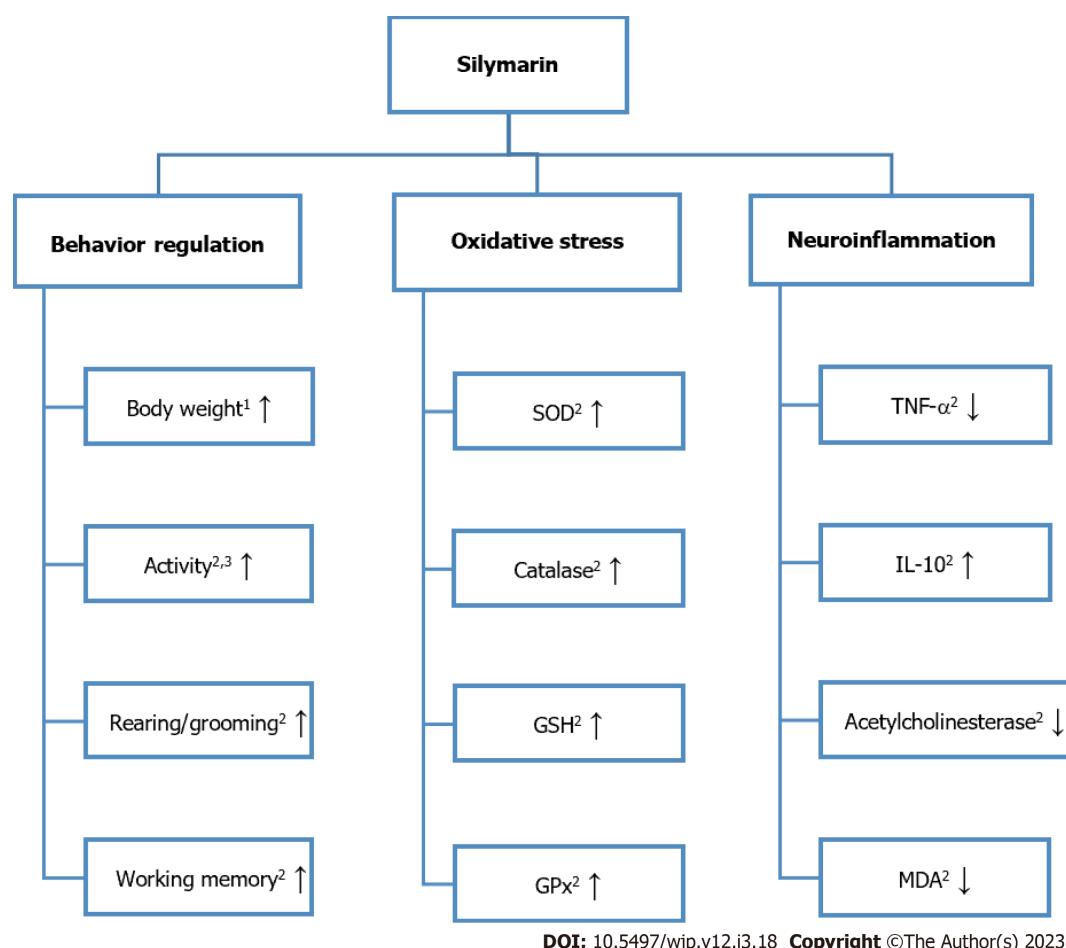


Figure 1 Effects of silymarin alone on the pathogenesis and symptoms of depression in a study on dexamethasone-induced mice.

¹Indicates significant difference in the group administered silymarin 280 mg/kg only. ²Indicates significant difference in both silymarin 140 mg/kg and 280 mg/kg.

³Activity includes tail suspension and forced swim tests, elevated plus maze, and locomotor activity. ↑ Indicates a significant increase ($P < 0.05$) relative to dexamethasone-induced mice. ↓ Indicates a significant decrease ($P < 0.05$) relative to dexamethasone-induced mice. GPx: Glutathione peroxidase; GSH: Glutathione; IL-10: Interleukin-10; MDA: Malondialdehyde; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor-alpha.

administered with sertraline and DEX or alone with DEX attenuated the DEX-induced reduction in working memory. They also found that SILY was able to positively affect anxiety-related behaviors tested using the elevated plus maze. The results demonstrated that SILY alone increased the amount of time mice spent in the open arm and decreased the amount of time spent in the closed arm. SILY alone with DEX or with sertraline and DEX also counteracted the DEX-induced changes in time. El-Elmat *et al* [35] similarly demonstrated that SILY administration can prevent stress-induced memory impairments and improve anxiety-related behaviors in a rat model of post-traumatic stress disorder.

Results from the tail suspension and forced swim tests showed that SILY alone reduced the amount of immobility time significantly when compared to the vehicle control and sertraline groups. Further, SILY by itself or with sertraline attenuated the DEX-induced increases in immobility time in both tests. Lee *et al* [36] also assessed immobility time in forced swim tests in a rat model of stress and found a significant decrease in immobility time with administration of the main active ingredient in SILY.

SILY's effects on antioxidant status, inflammatory markers, and neuronal injury

Onaolapo *et al* [1] found mixed results for SILY's effect on serum antioxidant status. Serum SOD levels increased significantly with both SILY concentrations and with DEX and high dose SILY. A decrease in serum SOD levels was found for DEX, DEX with sertraline, and both concentrations of SILY administered with DEX and sertraline. These results are interesting given that multiple studies have found increased SOD in patients with major depressive disorder [29,37-39]. With regards to catalase, serum concentrations increased significantly with both concentrations of SILY and DEX administered with SILY 280 mg/kg when compared to the vehicle control. Surprisingly, DEX with sertraline and DEX with SILY 140 mg/kg and sertraline resulted in a significant decrease in catalase serum concentrations when compared to the vehicle control. GSH also increased significantly with both concentrations of SILY and with DEX and SILY 280 mg/kg and DEX with SILY 280 mg/kg and sertraline when comparing to the vehicle control. The final antioxidant status measure, GPx, was found to have increased significantly with both concentrations of SILY and decrease with DEX and DEX with

sertraline compared to the vehicle control. These mixed results put SILY's effect on antioxidant status into question. However, another study by Ashraf *et al*[34] investigated SILY's antioxidant potential in a chronically induced stress model of depression and found catalase, GSH, and SOD levels from both the hippocampus and cerebral cortex to be significantly greater in mice treated with SILY 200 mg/kg as compared to the control.

Inflammatory marker levels from the hippocampus and cerebral cortex were improved following SILY administration. TNF- α levels were lowered in mice administered SILY alone. Further, results showed SILY's ability to mitigate the severely lowered TNF- α levels observed in DEX mice. IL-10 levels were increased in mice administered SILY alone. Results also demonstrated SILY's ability to mitigate the decrease in IL-10 levels observed in DEX mice. Acetylcholinesterase activity was found to be significantly increased in DEX mice; however, this increase was again mitigated by SILY administration alone or in conjunction with sertraline. SILY at both concentrations also significantly reduced acetylcholinesterase activity as compared to the control and sertraline. Both concentrations of SILY were also found to significantly reduce the levels of MDA as compared to the control. SILY 280 mg/kg was also found to significantly reduce the levels of MDA when compared to sertraline, as well. As with the other inflammatory markers, SILY alone or with sertraline attenuated the increase in MDA levels observed with DEX administration. The levels of the brain antioxidant enzymes tested, GSH and GPx, were both increased with both concentrations of SILY when compared to control and to sertraline. Further, SILY alone or with sertraline also mitigated the decrease in GSH and GPx induced by DEX. Ashraf *et al*[34] similarly found improved levels of inflammatory markers in stressed mice, with significantly lowered IL-1 β and TNF- α compared to the control. Thakare *et al*[40] also found significantly lowered IL-6, MDA, and TNF- α in stressed mice when compared to the control. Onaolapo *et al*[1] finally examined the morphology of the cerebral cortex and hippocampus. Cerebral cortex and hippocampal histomorphology demonstrated DEX-induced neuronal injury; however, when mice were administered DEX, sertraline, and SILY the histology appeared to have normalized suggesting SILY and sertraline's potential neuronal protective effects.

CONCLUSION

Overall, Onaolapo *et al*[1] conducted a well-designed study, which elucidated the effects of SILY on depressive and anxiety related behaviors, neuroinflammation, oxidative stress, and neuronal injury. The number of groups, randomization schemes, and the inclusion of 10 animals per group indicates strong experimental design. Results regarding its effects on oxidative stress were unclear, however, existing literature does suggest SILY's ability to modulate oxidative stress[34,40]. As the authors pointed out, the lack of assessment of glucocorticoid levels limits the study's ability to determine SILY's impact on the hypothalamus-pituitary-adrenocortical axis, which is implicated in depression and anxiety[41]. Further, the authors did not study SILY's effects on monoamine levels in the brain and failed to comment on the sex-dependent effects of SILY, which have been demonstrated in a couple of studies[42,43]. Lastly, as this is an animal study, the results cannot reliably predict SILY's effects in humans.

Alternative solution

Another widely studied phytochemical, curcumin, has both nonclinical and clinical evidence suggesting its antidepressant potential[44]. Curcumin is main component of the spice turmeric or *Curcuma longa*, and like SILY, has been shown to have anti-inflammatory, antioxidant, and neuroprotective activities [45,46]. The literature has demonstrated curcumin's ability to increase levels of monoamines in the central nervous system (CNS), inhibit glutamate release, decrease inflammation, improve oxidative stress, and attenuate symptoms associated with depression and anxiety[44].

A study by Bhutani *et al*[47] found that curcumin administration significantly reversed the increased depressive behaviors, low CNS monoamine levels, and increased monoamine oxidase activity observed in rats subjected to chronic unpredictable stress. Lin *et al*[48] showed curcumin's ability to inhibit glutamate release in the prefrontal cortex of rats through the suppression of presynaptic voltage-gated calcium channels. Given that glutamate has been demonstrated to be elevated in patients with depression, these results suggest that curcumin inhibited glutamate release may provide antidepressant effects. Curcumin has also been shown to decrease inflammatory markers in a rat model of depression. Fan *et al*[49] found that pretreatment with curcumin repressed inflammatory processes including microglia activation and overexpression of IL-1 β , IL-6, and TNF- α which were induced by chronic unpredictable stress. Further, in a 2015 clinical trial, Yu *et al*[50] found that chronic supplementation with curcumin decreased inflammatory cytokines, IL-1 β and TNF- α , and salivary cortisol in depressed patients when compared to placebo. Naqvi *et al*[51] showed in an animal model that curcumin administration also improved oxidative stress, which is associated with depression[20]. This study found that curcumin administration in mice exposed to unpredictable chronic mild stress resulted in a reduction in depression and anxiety symptoms, lipid peroxidation, and antioxidant enzymatic activity. Similarly, Moradi Vastegani *et al*[52] demonstrated that curcumin pretreatment in mice administered lipopolysaccharide (LPS) resulted in significantly increased activity of SOD and GPx enzymes. Further, it

attenuated the LPS-induced anxiety and depressive behavior.

Future directions

An interesting application of the results from Onaolapo *et al*[1] could be a study on the potential synergistic effects of SILY and curcumin for depression. The combination of SILY and curcumin has been tested in both human colorectal cancer cell lines and in radiation induced kidney injury in rats[53, 54]. One study by Montgomery *et al*[53] found that curcumin sensitized SILY's effects in human colorectal cancer cell lines. Another study found the combination of curcumin and SILY for treatment of radiation induced kidney injury in rats to be potentially more effective than curcumin or SILY alone [54]. Based on the anti-inflammatory and antioxidant properties of both SILY and curcumin alone for depression and anxiety, and the combined effects tested in other disease states, combination therapy with SILY and curcumin may be a promising treatment with greater antidepressant potential. Further, in patients for whom SSRIs are not effective or are not preferred, this alternative combination treatment may offer benefit.

FOOTNOTES

Author contributions: Manhard CE and Lucke-Wold B both designed and wrote this minireview (commentary).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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Country/Territory of origin: United States

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S-Editor: Wang JJ

L-Editor: A

P-Editor: Ji MX

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Basic Study

In silico insight into Amurensinine - an N-Methyl-D-Aspartate receptor antagonist

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Specialty type: Pharmacology and pharmacy

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Chen X, United States; Emran TB, Bangladesh

Received: March 1, 2023

Peer-review started: March 1, 2023

First decision: April 13, 2023

Revised: May 5, 2023

Accepted: May 31, 2023

Article in press: May 31, 2023

Published online: June 16, 2023



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Abstract

BACKGROUND

Some isopavines can exhibit important biological activity in the treatment of neurological disorders since it is considered an antagonist of the specific N-methyl-D-Aspartate (NMDA) receptor. Amurensinine is an isopavine which still has few studies. In view of the potential of isopavines as NMDA receptor antagonists, theoretical studies using bioinformatics were carried out in order to investigate whether Amurensinine binds to the NMDA receptor and to analyze the receptor/Ligand complex. This data can contribute to understanding of the onset of neurological diseases and contribute to the planning of drugs for the treatment of neurological diseases involving the NMDA receptor.

AIM

To investigate the interaction of the antagonist Amurensinine on the GluN1A/GluN2B isoform of the NMDA receptor using bioinformatics.

METHODS

The three-dimensional structure of the GluN1A/GluN2B NMDA receptor was selected from the Protein Data Bank (PDB) - PDB: 4PE5, and the three-dimensional structure of Amurensinine (ligand) was designed and optimized using ACD/Schemsketch™ software. Prediction of the protonation state of Amurensinine at physiological pH was performed using MarvinSketch software (ChemAxon). Protonated and non-protonated Amurensin were prepared using AutoDock Tools 4 software and simulations were performed using Autodock Vina v.1.2.0. The receptor/Ligand complexes were analyzed using PyMol (Schrödinger, Inc) and BIOVIA Discovery Studio (Dassault Systemes) software. To evaluate the NMDA receptor/Amurensinine complex and validate the molecular

docking, simulations using NMDA receptor and Ifenprodil antagonist were performed under the same conditions. Ifenprodil was also designed, optimized and protonated, under the same conditions as Amurensinine.

RESULTS

Molecular docking simulations showed that both non-protonated and protonated Amurensinine bind to the amino terminal domain (ATD) domain of the GluN1A/GluN2B NMDA receptor with significant affinity energy, -7.9 Kcal/mol and -8.1 Kcal/mol, respectively. The NMDA receptor/non-protonated Amurensinine complex was stabilized by 15 bonds, while the NMDA receptor/protonated Amurensinine complex was stabilized by less than half, 6 bonds. Despite the difference in the number of bonds, the variation in bond length and the average bond length values are similar in both complexes. The complex formed by the NMDA receptor and Ifenprodil showed an affinity energy of -8.2 Kcal/mol, a value very close to that obtained for the NMDA receptor/Amurensinine complex. Molecular docking between Ifenprodil and the GluN1A/GluN2B NMDA receptor demonstrated that this antagonist interacts with the ATD of the receptor, which validates the simulations performed with Amurensinine.

CONCLUSION

Amurensinine binds to the NMDA receptor on ATD, similar to Ifenprodil, and the affinity energy is closer. These data suggest that Amurensinine could behave as a receptor inhibitor, indicating that this compound may have a potential biological application, which should be evaluated by *in vitro* and preclinical assays.

Key Words: Amurensinine; Bioinformatics analysis; Isopavines; Molecular docking; N-methyl-D-Aspartate receptor

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Core Tip: Amurensinine binds to a region of the amino terminal domain on the N-methyl-D-Aspartate receptor and the interaction is stabilized mainly by covalent bonds, which confer an affinity energy of significant value to the receptor/Ligand complex. The interaction between Amurensinine and the receptor, which is involved in neurological diseases, suggests that this isopavine may interfere with its function, so it may have therapeutic potential in this area.

Citation: Façanha Wendel C, Hapuke Oliveira Alencar Q, Viana Vieira R, Teixeira KN. In silico insight into Amurensinine - an N-Methyl-D-Aspartate receptor antagonist. *World J Pharmacol* 2023; 12(3): 25-34

URL: <https://www.wjgnet.com/2220-3192/full/v12/i3/25.htm>

DOI: <https://dx.doi.org/10.5497/wjp.v12.i3.25>

INTRODUCTION

Ion receptors are voltage-dependent ion channels which, when activated by an electrical potential difference, lead to the influx and/or efflux of ions. These receptors are classified into three main families: The N-Methyl-D-Aspartate (NMDA) receptors, the alpha-amino-3-hydroxy-methyl-5-4-isoxazolpropionic receptors and, the Kainate receptors[1].

Studies show that hyperactivation, inhibition or dysfunction of these receptors are related to the development of several diseases such as schizophrenia, stroke, Alzheimer disease[2], anti-NMDA receptor encephalitis[3] and depression[4]. Alterations in the NMDA receptor may also be associated with neural disorders such as epileptic aphasia and mental retardation[5,6].

During the resting potential, the NMDA receptor presents its subunits joined together[7-10] and its ion channel is blocked by Mg^{2+} ion. Depolarization of the postsynaptic membrane potential[1] removes the Mg^{2+} ion from the receptor channel entrance[11,12] and thus, the agonists Glycine and Glutamate are able to bind to the GluN1[13-16] and GluN2 subunits of the NMDA receptor, respectively[14,16].

The channel only returns to its normal state after the ligand binding domain (LBD) gap opens allowing the agonist to dissociate from the active site. The opening of the gap may be caused due to the binding of the agonist itself to the binding site; this triggers a conformational change that decreases the sensitivity of the active site[17-22].

Excitotoxicity is caused by excessive stimulation by neurotransmitters which can lead to cell damage or death. It may occur in events that characterize a central nervous system trauma, an ischemic or

hemorrhagic condition, in which cells are deprived of energy to maintain ionic homeostasis[23]. The excess of Glutamate in the medium facilitates the activation of the NMDA receptor which allows the flux of Ca^{2+} ions into the cell. This intracellular Ca^{2+} accumulation can cause an osmotic swelling, lysis and cell death[23], activating enzymes such as proteases, phospholipases, and endonucleases, which can damage cell structures such as membranes and DNA itself.

In this situation, mitochondria are also harmed, because they are unable to buffer this excess of Ca^{2+} , resulting in the formation of reactive oxygen species[23]. Neurons, which are positively charged (depolarized), promote the unblocking of the ion channel caused by Mg^{2+} , facilitating a greater influx of ions through the channel, making it difficult to reestablish ionic homeostasis[24,25].

Allosteric modulators that bind to the amino terminal domain (ATD) are able to regulate the probability of receptor ion channel opening and its rate of closure, such as Zn^{2+} which binds to the GluN2A and GluN2B subunits, Ifenprodil that binds to GluN2B, and polyamines that bind to GluN2B [26-28]. LBD also has binding sites for agonist or antagonist allosteric modulators, capable of controlling ion channel opening[29,30].

Ifenprodil is a non-competitive antagonist that partially binds to the ATD of the GluN2B subunit of the NMDA receptor - where the Glutamate agonist binding site is located, and can inhibit its activity by up to 90% showing higher efficiency at GluN1/GluN2B compared to GluN1/GluN2A/GluN1/GluN2B [31-33].

Isopavines are alkaloids derived from plants of the genus *Papaver*[34-37] and are classified as benzopyridine isoquinolines[37] that have in common the tetrahydroisoquinoline in the central region of their structures[38,39]. Isopavines are considered non-competitive and specific NMDA receptor ion channel antagonists or blockers[39]. Studies indicate that isopavines may exhibit important biological activity in the treatment of neurological disorders such as Down syndrome, Alzheimer, Huntington's disease, amyotrophic lateral sclerosis, senile dementia, stroke, epilepsy, and olivo-ponto-cerebellar atrophy[38,39]. Amurensinine is an isopavine that has been found in *Meconopsis* species[40] and it has been identified in *Papaver alpinum*, *Papaver tatricum*, *Papaver pyrenaicum*, *Papaver suaveolens* and some varieties of *Papaver nudicaule*[34-37].

Molecular docking is a computational method used to predict possible sites of interaction of a ligand in a receptor, as well as the affinity of the interaction between receptor-ligand, the conformation of the receptor-ligand complex and the nature of the chemical bonds between the receptor and the ligand in order to define the stability of the interaction. This method has great importance for the development of new drugs, because it allows you to optimize the design of a drug, to find a candidate drug by virtual scanning in databases[41].

Therefore, molecular docking simulations between Amurensinine and the NMDA receptor were performed in order to evaluate the receptor/Ligand complex, and thereby provide data to support future research on neurological diseases involving this receptor.

MATERIALS AND METHODS

Receptor and ligands

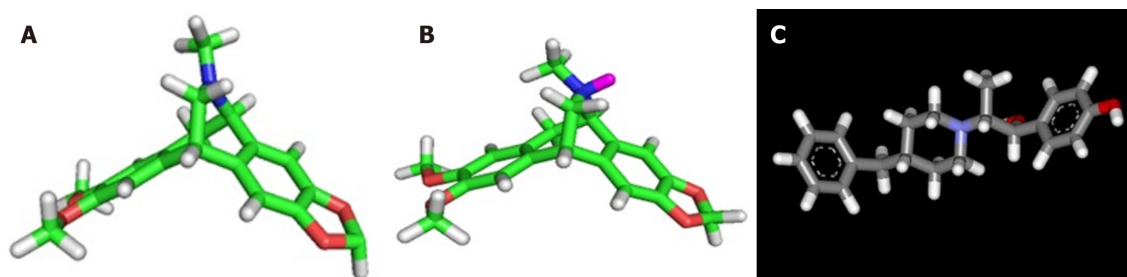
This study was performed with the GluN1A/GluN2B isoform of the NMDA receptor. The native mutation-free three-dimensional structure of the human GluN1A/GluN2B NMDA receptor is not available on the structure databases, thus the structure of the receptor from a mammal used in preclinical trials - *Rattus norvegicus* - was selected. The three-dimensional structure of the GluN1A/GluN2B NMDA receptor was selected from the Protein Data Bank (PDB) (www.rcsb.org) - PDB 4PE5. Three-dimensional structure of Amurensinine (ligand) was designed and optimized using ACD/Schemsketch™ software. The protonation state prediction of the Amurensinine at physiological pH 7.4 was performed using the MarvinSketch software (ChemAxon). Molecular docking simulations were performed with Amurensinine in its protonated and non-protonated states.

Molecular docking and data analyses

Molecular docking was performed using the flexible ligand - rigid receptor methodology[42]. Simulations were performed with the B-chain of the GluN2B subunit of the GluN1A/GluN2B NMDA receptor, where the ATD - domain in which the binding site of the antagonist Ifenprodil, is located in human receptor. For the execution of molecular docking, the AutoDock Tools 4[43] and Autodock Vina v.1.2.0.[44] software was used. In these software the steps for the preparation of molecules were performed, the torsion points of ligand were detected, its torsion angles were calculated and Grid box dimensions were determined and the command was run by AutoDock Vina. Data were analyzed using PyMol (Schrödinger, Inc) and BIOVIA Discovery Studio (Dassault Systemes) software.

Molecular docking validation

To validate and analyze the NMDA receptor/Amurensinine complex, the molecular docking was performed with the Ifenprodil antagonist (Figure 1 under the same conditions. Ifenprodil was also designed, optimized and protonated as well as Amurensinine. Ifenprodil was docked on the ATD of the



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Figure 1 Structures of Amurensinine and Ifenprodil. A: Amurensinine in the non-protonated state; B: Amurensinine in the protonated state at pH 7.4; C: Ifenprodil. These structures were designed using ACD/Schemsketch software.

GluN1A/GluN2B NMDA receptor (PDB 4PE5).

RESULTS

At physiological pH (pH = 7.4) it was observed that 68.5% of Amurensinine was in its non-protonated state and 31.5% was in the protonated one; both states show structural differences (Figure 1). Molecular docking showed that Amurensinine binds to the NMDA receptor; both protonated and non-protonated Amurensinine binds to the ATD of the GluN1A/GluN2B NMDA receptor with significant affinity energy, -7.9 Kcal/mol and -8.1 Kcal/mol, respectively. Despite the similar affinity energy values, the non-protonated Amurensinine makes 15 bonds with regions of the ATD, while the protonated compound makes only six bonds (Table 1). However, the variation in bond lengths and the average of these lengths in both the non-protonated Amurensinine/NMDA receptor complex (2.9-5.4 Å; average = 4.23) and the protonated Amurensinine/NMDA receptor complex (2.0-5.7 Å; average = 3.90) are similar. Upon coupling of Ifenprodil to ATD, an energy affinity = -8.2 Kcal/mol was obtained, a value very close to that obtained for protonated Amurensinine/GluN1A/GluN2B NMDA receptor complex; even the same number of bonds was observed (six bonds) (2.6-4.9 Å; average = 3.67) as shown in Table 2; however, the geometry of the bonds is different (Figure 2). This result is consistent, since the structures of Amurensinine and Ifenprodil are similar, but not identical (Figure 3). The interaction between Ifenprodil and ATD on NMDA receptor validates the molecular docking of Amurensinine and confers reliability to the data.

DISCUSSION

Neurological diseases are an important cause of morbidity and mortality and loss of quality of life. Their pathophysiology is complex and multifactorial, since it involves genetic and environmental factors, resulting in different clinical manifestations in patients with the same neurological damage[45].

Elucidated mechanisms involved in the onset of neuronal diseases include hyperactivation, inhibition and dysfunction of the NMDA receptor, which is involved in schizophrenia, stroke, Alzheimer[2], anti-NMDAR encephalitis[3], depression[4], mental retardation and epileptic aphasia[5,6]. This receptor is an ion channel composed of four subunits and has three families - GluN1, GluN2 (A-D isoforms) and GluN3 (A-B isoforms)[46]. Each receptor subunit is composed of two extracellular domains - LBD and ATD, an intracellular carboxy terminal domain (CTD) and a transmembrane domain (TMD), where the ion channel is located[7,16,39,47-49].

Therefore, compounds which regulate the opening and closing speed of the ion channel by binding to ATD, are promising for research into the treatment of neurological diseases[39,49]. *In silico*, the Amurensinine binds to the ATD of the GluN1A/GluN2B NMDA receptor with significant affinity. The affinity is measured by affinity energy which, when it is lower than -6.0 kcal/mol, indicates interaction in a biological environment[50]. This data is important once it indicates the possibility of interaction of the Amurensinine with the target receptor inside the organism. Ifenprodil is known to bind to the GluN2B subunit of the human NMDA receptor[32]; by binding in the same region of the NMDA receptor from *Rattus norvegicus* as Amurensinine, it is suggested that this isopavine may also interact with the human receptor. In addition, the low value of affinity energy points out the stability of the Amurensinine/NMDA receptor complex, and consequently indicates chances of pharmacological effectiveness. However, these assumptions need to be tested experimentally, *in vitro* and *in vivo*.

NMDA receptors normally function at physiological pH - neutral[44]; in cases of brain damage or neural disease, this pH may decrease and the environment becomes acidic[44,45]. The interaction of Amurensinine with the NMDA receptor in both protonated and non-protonated states, with similar

Table 1 Chemical bonds of the GluN1A/GluN2B N-methyl-D-Aspartate receptor/Amurensinine complex

Non protonated Amurensinine: Receptor ¹	Distance (Å)	Bond type
Aromatic ring (A): ARG347	4.1	π -alky ¹
Aromatic ring (A): TYR 287	4.8	π - π T-shaped
Aromatic ring (B): LYS361	2.9	Hydrogen bond
Aromatic ring (B): LYS361	4.8	π -alky ¹
Aromatic ring (B): PRO360	5.4	π -alky ¹
C-12: ARG347	4.2	Alky ¹
C-12: LEU349	5.2	Alky ¹
C-12: TYR287	5.1	π -alky ¹
C-22: ASP348	3.6	Carbon-hydrogen bond
C-9: PHE146	3.4	Carbon-hydrogen bond
Cycloheptane: LYS361	4.8	Alky ¹
Cyclopentane: LYS361	3.3	Hydrogen bond
Cyclopentane: PRO360	5	π -alky ¹
N-1: HIS359	3.3	Hydrogen bond
O-3: ASP295	3.5	Hydrogen bond
Protonated Amurensinine: Receptor*	Distance (Å)	Bond type
Aromatic ring (B): ASP477	4.4	π -anion
Aromatic ring (B): TRP166	5.7	Alky ¹
CH-19: TYR476	3.1	Carbon-hydrogen bond
Cyclopentane: VAL390	4.9	π - alky ¹
NH-1: ASP477	2	Carbon-hydrogen bond
O1: ASP165	3.3	Hydrogen bond

¹Atom: Residue.

Amino acid residues are shown in the three-letter code and the number represents the position of the residue in the polypeptide chain. (A) and (B) indicate the different aromatic rings of Amurensinine.

Table 2 Chemical bonds in the GLUN1A/GLUN2B N-methyl-D-Aspartate receptor/lfenprodil complex

lfenprodil: Receptor ¹	Distance (Å)	Bond type
Aromatic ring: ARG393	3.8	π -sigma
Aromatic ring: VAL390	4.6	Alky ¹
Aromatic ring: LYS454	4.9	π - alky ¹
N-1: TYR164	3.5	Hydrogen bond
OH-2: THR255	2.6	Hydrogen bond
OH-1: ASP477	2.6	Hydrogen bond

¹Atom: Residue.

Amino acid residues are shown in the three-letter code and the number represents the position of the residue in the polypeptide chain.

affinities, indicate that the pH and, consequently, the protonation state of this compound, do not influence its interaction. Thus, neurological disorders that alter the H⁺ concentration in the environment will not have a significant influence on the modulating activity of Amurensinine on the receptor.

An opposite result was observed with Endobain E and the NMDA receptor. This compound is an endogenous brain factor that acts as an inhibitor of Na/K ATPase and, as a modulator of the NMDA receptor by binding to the inner surface of the channel, decreasing the affinity of receptor ligands.

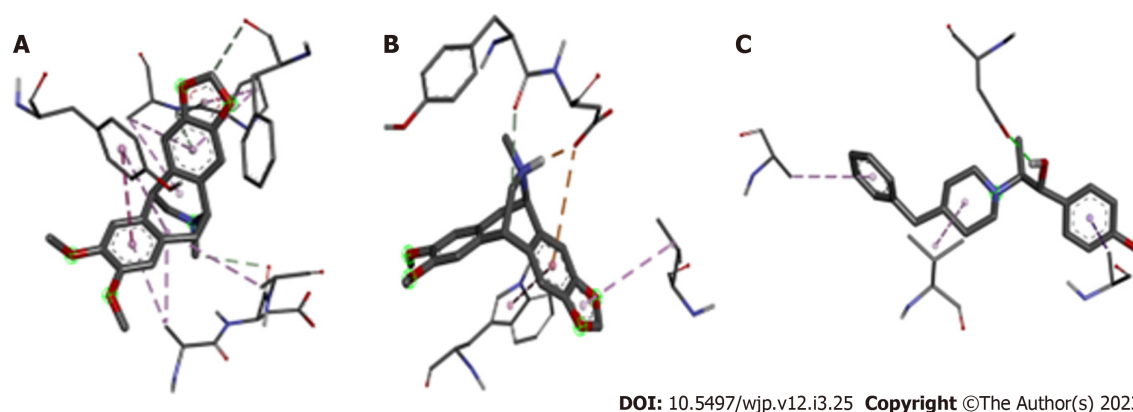


Figure 2 Binding geometry of antagonists to the amino terminal domain on the GluN1A/GluN2B N-methyl-D-Aspartate receptor. A: Non-protonated Amurensinine; B: Protonated Amurensinine; C: Ifenprodil. The structures in thick sticks are the antagonists; the thin sticks are the amino acid residues of the receptor. Chemical bonds are represented by dashed lines. These structures were designed using ACD/Schemsketch software.

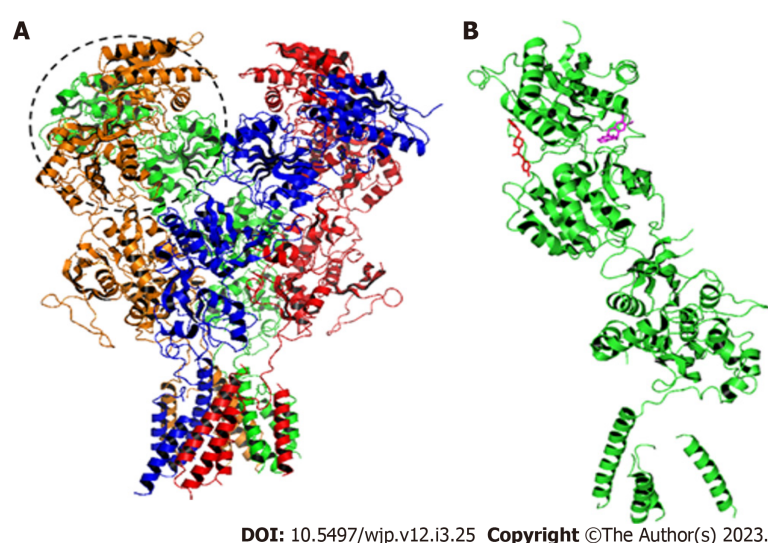


Figure 3 Structure of the GluN1A/GluN2B N-methyl-D-Aspartate receptor. A: The dashed circle indicates the amino terminal domain (ATD). A-Chain: Orange; B-Chain: Green; C-Chain: Red; D-Chain: Blue. In the B-Chain are located the binding sites for Amurensinine and Ifenprodil; B: B-Chain with Ifenprodil (red) and non-protonated Amurensinine (pink) bound to the ATD. These structures were designed using ACD/Schemsketch software.

Under conditions of cerebral ischemia, Endobain E has its activity optimized due to the acidic environment of the ischemic brain (pH 6.5, 90% receptor inhibition). In a more alkaline environment, its activity is reduced (pH 7.4-8, 25% receptor inhibition)[51].

In general, proteins such as NMDA receptor-forming ones, have their structural conformation and, consequently, their activity directly influenced by pH. The sudden change in H⁺ concentration induces ionization of the residues, conferring an excessively negative or positive charge, which can lead to intramolecular repulsion, exposure of hydrophobic regions, and loss of function[52]. On the other hand, pH variation can also expose previously hidden sites, optimizing the receptor-ligand interaction, as occurs with Endobain E. Regarding Amurensinine, it is inferred that its binding site is not in a region of the receptor that undergo major conformational changes with pH variation of the medium and that this site is capable of binding both the protonated and non-protonated states of this isopavine.

The binding strength, also called intermolecular interaction, occurs between covalently interacting compounds and determines the effectiveness of the receptor-ligand interaction[53]. Moreover, the bond type that molecules establish with each other directly influences their physicochemical properties, such as solubility and boiling point, and their final conformation. In enzymes the type of contact established is able to alter their activity, strength, selectivity and the conformation of the enzyme-substrate complex [54]. Thus, predicting the strength and type of interaction between receptor and ligand is important to understand the mechanism of action of the ligand, which may be a drug candidate.

Amurensinine in protonated state interacts with the receptor through six chemical bonds of which the majority (83%) are covalent bonds. Amurensinine in the non-protonated state made 15 chemical bonds with the receptor, with a predominance of covalent bonds, and even with the large difference in the

number of bonds, the affinity energy was quite similar for both receptor/Ligand complexes. Possibly, a contributing factor to the similarity of affinities is the shorter bond lengths that stabilize the protonated Amurensinine/receptor complex.

In this regard, Medeiros[55] cites the physicochemical characteristics of the interaction of antagonistic compounds, such as Ifenprodil, with the ATD in the GluN2B subunit of the NMDA receptor. This compound, despite having a strong interaction with the receptor, has little selectivity as it also binds to the Alpha-1 and 5HT receptors, leading to impairments in motor function and a drop in blood pressure. However, its use is of fundamental importance as a parameter for studying compounds that interact differently with the binding site, such as MK-22, EVT-101, and Amurensinine[55].

CONCLUSION

Amurensinine, in both protonated and non-protonated states, binds to the ATD region of the GluN2B subunit of the murine GluN1A/GluN2B NMDA receptor, *in silico*, forming complexes stabilized mainly by covalent bonds. The affinity energy of the complexes is significantly low and indicates, in addition to probability of interaction in the physiological environment, the stability of the complexes. The structural and receptor interaction similarity between Amurensinine and Ifenprodil suggest that this isopavine could behave as a receptor inhibitor; therefore, this compound could present potential biological application, which needs to be evaluated by *in vitro* and *in vivo* assays.

ARTICLE HIGHLIGHTS

Research background

Isopavines are alkaloids derived from plants of the genus *Papaver* that have biological activity for the treatment of neurological disorders. Amurensinine is a little-studied isopavine whose activity in relation to neurological disorders has not been studied. Research on this isopavine may contribute new information about its action.

Research motivation

The research motivation was the scarce literature on the subject. There are few studies in health care using isopavines; similarly, studies on Amurensinine are quite scarce. Therefore, we studied Amurensinine *in silico* to verify its preliminary therapeutic potential.

Research objectives

To study, *in silico*, the interaction between Amurensinine and the N-methyl-D-Aspartate (NMDA) receptor, which is involved in the onset of neurological disorders.

Research methods

In this study we used molecular docking, a standardized bioinformatics methodology for analyzing chemical interactions between receptors and ligands.

Research results

The research results indicated that Amurensinine can interact with the NMDA receptor with high affinity, and that its protonation state does not significantly interfere with this affinity.

Research conclusions

The results of the research were satisfactory, as Amurensinine was able to bind to the tNMDA receptor. The occurrence of interaction indicates that this isopavine may interfere with the activity of the receptor. Since it binds with similar affinity and to the same subunit as the antagonist Ifenprodil, it may act as an inhibitor of the receptor. However, *in vitro* and *in vivo* studies are needed to affirm this.

Research perspectives

The research perspective is to conduct further *in silico* analyses with Amurensinine and other receptors involved in the onset of neurological diseases to further evaluate the potential of this isopavine for health care.

FOOTNOTES

Author contributions: Façanha Wendel C performed the experiments and analyzed the results; Hapague Oliveira

Alencar Q and Viana Vieira R wrote the manuscript; Teixeira KN interpreted the data, performed the critical analysis of the results and coordinated the study; All authors approved the final version of the manuscript.

Institutional review board statement: The study was conducted *in silico* and involved neither humans nor animals, so it was not necessary Institutional review board statement.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The study was conducted only in a computational environment and the data and three-dimensional structures used are available in public online databases.

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S-Editor: Li L

L-Editor: A

P-Editor: Ji MX

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World Journal of *Pharmacology*

World J Pharmacol 2023 December 22; 12(4): 35-52



ORIGINAL ARTICLE

Basic Study

- 35 Natural isothiocyanates of the genus *Capparis* as potential agonists of apoptosis and antitumor drugs
Hanuš L, Naor T, Glorizova T, Dembitsky VM

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INDEXING/ABSTRACTING

The WJP is now abstracted and indexed in Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: Hua-Ge Yu; Production Department Director: Xiang Li; Editorial Office Director: Yun-Xiao Jiao Wu.

NAME OF JOURNAL

World Journal of Pharmacology

ISSN

ISSN 2220-3192 (online)

LAUNCH DATE

February 9, 2012

FREQUENCY

Continuous Publication

EDITORS-IN-CHIEF

Pharkphoom Panichayupakaranant, Ahmed M Kabel, Muhammad Shahzad

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<https://www.wjnet.com/2220-3192/editorialboard.htm>

PUBLICATION DATE

December 22, 2023

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Basic Study

Natural isothiocyanates of the genus *Capparis* as potential agonists of apoptosis and antitumor drugs

Lumír Hanuš, Tuvia Naor, Tatyana Gloriovova, Valery M Dembitsky

Specialty type: Pharmacology and pharmacy**Provenance and peer review:**

Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind**Peer-review report's scientific quality classification**

Grade A (Excellent): A

Grade B (Very good): 0

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

P-Reviewer: Gupta S, Brazil; Zhang J, China**Received:** August 28, 2023**Peer-review started:** August 28, 2023**First decision:** September 19, 2023**Revised:** October 12, 2023**Accepted:** November 24, 2023**Article in press:** November 24, 2023**Published online:** December 22, 2023**Lumír Hanuš**, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University, Ein Kerem Campus, Jerusalem 91120, Israel**Tuvia Naor**, Food Chemistry, Kibbutz, Yotvata 8882000, Israel**Tatyana Gloriovova**, Department of Bioinformatics, Institute of Biomedical Chemistry, Moscow 119121, Russia**Valery M Dembitsky**, Centre for Applied Research and Innovation, Lethbridge College, Lethbridge AB T1K 1L6, Canada**Corresponding author:** Lumír Hanuš, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University, Ein Kerem Campus, Jerusalem 91120, Israel.lumirh@ekmd.huji.ac.il

Abstract

BACKGROUND

Using gas chromatography-mass spectrometry (GC/MS) analysis, we examined the composition of volatile components present in the yellow and green fruits, seeds, and jam of the scrambling shrub *Capparis cartilaginea* (*C. cartilaginea*). These plant samples were collected from Kibbutz Yotvata in Israel. In all the tested samples, isothiocyanates were identified. Utilizing the PASS program, we ascertained the biological activity of these isothiocyanates present in the *Capparis* genus. The study results highlighted that all isothiocyanates could potentially act as apoptosis agonists, making them strong candidates for antitumor drugs. This information holds significant value for the fields of medicinal chemistry, pharmacology, and practical medicine.

AIM

To investigate the volatile components present in the yellow and green fruits, seeds, and jam of the *C. cartilaginea* shrub using GC/MS analysis, to detect isothiocyanates in all the analyzed plant samples, and to assess the biological activity of these isothiocyanates utilizing the PASS program.

METHODS

We utilized two primary methods to analyze the volatile compounds present in the yellow and green fruits, seeds, and jams of the *C. cartilaginea*, native to Israel. We identified biologically active isothiocyanates in these samples. Their antici-

pated biological activities were determined using the PASS program, with the most dominant activities being apoptosis agonist, anticarcinogenic, and antineoplastic specifically for genitourinary cancer.

RESULTS

Fruits, seeds, and jams containing isothiocyanates, which exhibit antineoplastic and anticarcinogenic activities, could be suggested for cancer prevention and management. Specific isothiocyanates, with therapeutic potential in this realm, could be recommended as potent anticancer agents in practical medicine following clinical trials.

CONCLUSION

The discovery that isothiocyanates exhibit potent antineoplastic and anticarcinogenic activities was unexpected. Additionally, certain isothiocyanates demonstrated antifungal, antiviral (specifically against arbovirus), and antiparasitic properties.

Key Words: *Capparis cartilaginea*; Fruits; Seeds; Isothiocyanates; Apoptosis; Anticancer

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Core Tip: Using gas chromatography-mass spectrometry analysis, we examined the composition of volatile components present in the yellow and green fruits, seeds, and jam of the scrambling shrub *Capparis cartilaginea*. Fruits, seeds, and jams containing isothiocyanates, which exhibit antineoplastic and anticarcinogenic activities, could be suggested for cancer prevention and management. Specific isothiocyanates, with therapeutic potential in this realm, could be recommended as potent anticancer agents in practical medicine following clinical trials.

Citation: Hanuš L, Naor T, Glorizova T, Dembitsky VM. Natural isothiocyanates of the genus *Capparis* as potential agonists of apoptosis and antitumor drugs. *World J Pharmacol* 2023; 12(4): 35-52

URL: <https://www.wjgnet.com/2220-3192/full/v12/i4/35.htm>

DOI: <https://dx.doi.org/10.5497/wjpv12.i4.35>

INTRODUCTION

The genus *Capparis*, part of the Capparidaceae family, comprises approximately 250 species[1-3]. The Cartilage caper is notably prevalent across tropical and subtropical regions in Asia, America, and Africa[4-6]. Recent findings suggest that the genus *Capparis* encompasses about 400 compounds, including glycosides, glucosinolates, flavonoids, terpenoids, tannins, steroids, and isothiocyanates[7,8]. There is substantial evidence indicating the therapeutic potential of these phytochemicals in treating and preventing various ailments such as inflammation, cancer, bacterial infections, ulcers, and diabetes[9-11].

This study explored the volatile compounds present in the yellow and green fruits, seeds, and jam of *Capparis cartilaginea* (*C. cartilaginea*), a species native to Israel. Furthermore, we were keen to examine the distribution of isothiocyanates in the essential oils of the *Capparis* genus from various global regions. Included is a table detailing the isothiocyanates identified within the *Capparis* genus, along with their anticipated biological activities as determined using the PASS software.

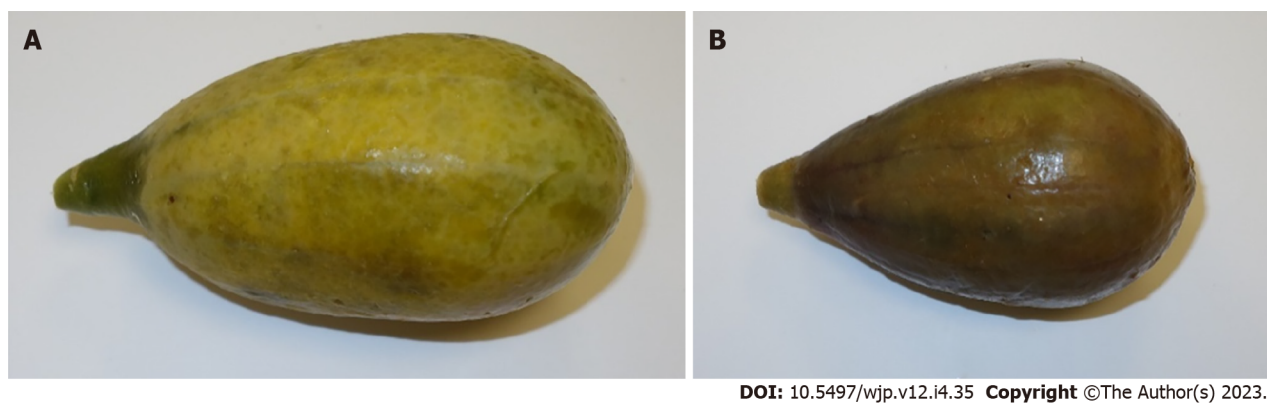
MATERIALS AND METHODS

Plant materials and extraction

The scrambling shrub *C. cartilaginea*, grown in Kibbutz Yotvata in Israel, was the source of the yellow and green fruits (Figure 1) harvested in 2019 for component analysis. The aromatic, juicy pulp of the fruit was available at the Kibbutz store, labeled as “Jam of Tuvia Naor”. Samples were taken from shrubs reaching heights of up to 3 meters. The fruit is globose-ellipsoid in shape, with a reddish hue, measuring (3-6) cm × (1.6-4) cm. Fresh biological materials underwent head space and solid phase microextraction gas chromatography-mass spectrometry (GC-MS) analysis, following the methods that we have previously detailed[12-14].

General experimental procedures

For the GC/MS analysis, we employed an Agilent 7890B GC combined with an Agilent 5977B MSD and a PAL 3 (RSI 85) chromatograph. The columns used were HP-5MS UI, 30 m × 0.25 mm with a film thickness of 0.25 µm, provided by Agilent Technologies, Inc. The analytical conditions were set with the column initially held at 35 °C for 5 min. Subsequently, the temperature was programmed to rise from 35 °C to 150 °C at a rate of 5 °C/min, then increasing by 15 °C/min to 250 °C, with a hold time of 90 min. The specific settings were as follows: Inlet temperature at 250 °C, detector



DOI: 10.5497/wjpv.v12.i4.35 Copyright ©The Author(s) 2023.

Figure 1 Yellow and green fruits harvested from the scrambling shrub *Capparis cartilaginea* contain a different set of components. A: Yellow fruit; B: Green fruit.

temperature at 280 °C, split injection ratio of 1:5, initial temperature at 100 °C, and initial time set to 4.0 min. Helium was used as the carrier gas with a flow rate of 1 mL/min.

For compound detection and identification, we referenced various standards, retention times, and retention indices (Table 1). Additionally, we consulted multiple libraries: NIST/EPA/NIH Mass Spectral Library 2017, Wiley Registry of Mass Spectral Data 11th Edition, FFNSC3, © 2015, and the Adams EO library, Mass Spectral Library, containing 2205 compounds. In total, 58 volatile compounds were detected, with 42 of them being positively identified. This identification was based on a comparison of their mass spectra and retention times, along with their Kovats retention indices, either to those of injected standards or by referencing the National Institute of Standards and Technology's Mass Spectral Library database.

Comparison of biological activities of natural isothiocyanates

The principle that the chemical structure of natural or synthetic molecules dictates their biological activity has been recognized for over 150 years and is referred to as structure-activity relationships (SAR). This concept was first introduced by Brown and Fraser[15] in 1868. However, according to alternate sources[16], the SAR notion was earlier employed in the realm of toxicology. In this context, Cros determined the correlation between the toxicity of primary aliphatic alcohols and their water solubility as early as 1863.

In this particular study, we sourced PASS predictions for approximately 28 isothiocyanates derived from various plants. These PASS estimates are represented as Pa values. Each Pa value signifies the likelihood of a compound being categorized under “actives” for a given predicted biological activity. A higher Pa value denotes greater confidence in the anticipated biological activity[17,18].

RESULTS

Various components from leaves, buds, stems, aerial parts, and seeds of different plant species within the *Capparis* genus have been documented in the literature. Yet, no literature data was found pertaining to the study of yellow and green fruits or jam derived from *C. cartilaginea*. Based on our GC/MS findings, the primary constituents of the yellow fruits were identified as 2-butyl isothiocyanate (49.43%) and isopropyl isothiocyanate (48.74%), as visualized in the chromatogram (Figure 2A). A similar compositional profile was observed for the green fruits, with the dominant components being 2-butyl isothiocyanate (49.76%) and isopropyl isothiocyanate (46.68%), as shown in Table 2 and illustrated in the chromatogram (Figures 2B and 3).

The GC/MS analysis of the seeds from *C. cartilaginea* revealed dimethylsulfide as the predominant component, constituting 55.82%, while the content of 2-butyl isothiocyanate was notably lower at just 6.8%. These findings can be referenced in Table 3 and visualized in the chromatogram (Figure 2C). Furthermore, the GC/MS analysis of jam derived from *C. cartilaginea* indicated that its primary components were hexanedioic acid bis(2-ethylhexyl) ester at 61.99%, limonene (covering both isomers) at 8.51%, dimethyl sulfide at 3.85%, 2-butyl isothiocyanate at 3.29%, dodecanoic acid 1-methylethyl ester at 2.16%, and pentanoic acid, 2-ethylhexyl ester at 2.01% (Figures 2D and Table 4). The molecular structures of these identified compounds are depicted in Figure 4. Tuvia Naor jam consists of the fruits of *C. cartilaginea* (or *Capparis inermis*, or a synonym for *Capparis sinaica*). Homemade jam Tuvia capparis Jam from the fruits of *C. cartilaginea* contains 36% fruit, sugar, apple, lemon, and flavors.

DISCUSSION

The experimental data reveals that all parts of plants from the *Capparis* genus contain isothiocyanates in varying concentrations. It was intriguing to discern which specific isothiocyanates were present in this genus. This curiosity stems from

Table 1 Composition of components that were identified from yellow fruits of *Capparis cartilaginea*

Peak	RT	Area	%	Compound	RI
1	2.477	67563.26	0.16	Isopropyl nitrile	623
2	4.145	48903.53	0.11	N-methylene-ethenamine	727
3	8.089	20864469.13	48.74	Isopropyl isothiocyanate	837
4	10.846	55386.82	0.13	2-butenyl isothiocyanate	887
5	11.832	21159669.73	49.43	2-butyl isothiocyanate	920
6	12.578	597488.12	1.40	Isobutyl isothiocyanate	926
7	25.268	17767.47	0.04	Benzyl isothiocyanate	1359

RT: Retention time; RI: Retention index.

Table 2 Composition of components that were identified from green fruits of *Capparis cartilaginea*

Peak	RT	Area	%	Compound	RI
1	1.756	283022.83	0.19	Dimethylsulfide	520
2	2.453	308033.24	0.21	Isobutyronitrile	626
3	3.864	23282.6	0.02	Sec-butyl cyanate	689
4	4.145	284696.04	0.19	N-methylene-ethenamine	727
5	6.991	10973.58	0.01	Ethyl isothiocyanate	796
6	8.193	68423753.03	46.68	Isopropyl isothiocyanate	837
7	10.59	6355.8	0.00	Propyl isothiocyanate	881
8	10.854	393825.2	0.27	2-butenyl isothiocyanate	887
9	11.929	72938834.61	49.76	2-butyl isothiocyanate	920
10	12.586	3848951.24	2.63	Isobutyl isothiocyanate	926
11	25.276	70246.54	0.05	Benzyl isothiocyanate	1359

RT: Retention time; RI: Retention index.

Table 3 Composition of components that were identified from seeds of *Capparis cartilaginea*

Peak	RT	Area	%	Compound	RI
1	1.779	557486.8	55.82	Dimethylsulfide	520
2	2.493	18043.82	1.81	Isobutyronitrile	626
3	2.822	41394.08	4.14	3-methylbutanal	652
4	2.958	31612.69	3.17	2-methyl-butanal	662
5	8.112	280745.6	28.11	Isopropyl isothiocyanate	837
6	11.84	67963.7	6.80	2-butyl isothiocyanate	920
7	12.618	1561.57	0.16	Isobutyl isothiocyanate	926

RT: Retention time; RI: Retention index.

the fact that isothiocyanates are invaluable plant metabolites known for their broad spectrum of biological activities. Notably, certain isothiocyanates are incorporated into Tibetan and Chinese medicinal practices[19-21]. These naturally occurring molecules originate from glucosinolate precursors found in cruciferous vegetables[19,22-25].

Tables 5 and 6 provide a quantitative breakdown of the distribution of isothiocyanates across different plant species within the *Capparis* genus, collected from various global regions. While many articles discuss isothiocyanates, not all

Table 4 Composition of components that were identified from jam of *Capparis cartilaginea*

Peak	RT	Area	%	Compound	RI
1	1.788	272484.92	3.85	Dimethyl sulfide	520
2	2.453	59609.49	0.84	Isobutyronitrile	626
3	2.814	96185.08	1.36	3-methyl-butanal	652
4	2.926	34760.26	0.49	2-methyl-butanal	662
5	4.161	145138.02	2.05	N-methylene-ethenamine	727
6	8.113	85740.89	1.21	Isopropyl isothiocyanate	837
7	11.824	232997.63	3.29	2-butyl isothiocyanate	920
8	12.61	6608.49	0.09	Isobutyl isothiocyanate	926
9	13.347	77903.33	1.10	β -pinene	979
10	13.981	7385.93	0.10	β -myrcene	991
11	14.806	8950.21	0.13	α -terpinene	1018
12	15.079	5640.69	0.08	P-cymene	1025
13	15.215	601965.53	8.51	Limonene	1030
14	15.296	13202.57	0.19	Eucalyptol	1032
15	16.249	112624.11	1.59	γ -terpinene	1060
16	17.219	7218.34	0.10	Terpinolene	1088
17	20.402	11884.14	0.17	α -terpineol	1189
18	25.261	57235.71	0.81	2-(2-butoxyethoxy)-ethanol acetate	1366
19	26.638	142269.27	2.01	Pentanoic acid, 2-ethylhexyl ester	1404
20	28.014	76710.10	1.08	1-dodecanol	1473
21	28.59	25321.22	0.36	Pentadecane	1500
22	30.152	13471.82	0.19	Diphenyl sulfide	1552
23	30.264	83985.94	1.19	Hexadecane	1600
24	30.45	11616.10	0.16	Octadecanal	1357
25	30.636	152595.56	2.16	Dodecanoic acid 1-methylethyl ester	1618
26	31.408	80674.69	1.14	2-propenoic acid dodecyl ester	1675
27	31.473	41016.71	0.58	Heptadecane	1700
28	31.547	53606.2	0.76	(1-methyldecyl)-benzene	1708
29	31.64	73991.74	1.05	(1-methyldecyl)-benzene	1735
30	32.189	9636.895	0.14	2-methyl-octadecane	1863
31	32.449	27402.23	0.39	Nonadecane	1900
32	33.491	19861.16	0.28	Hexadecanoic acid methyl ester	1926
33	33.732	28833.81	0.41	Hexadecanoic acid	1968
34	34.021	9930.393	0.14	Heneicosane	2100
35	37.182	4384622	61.99	Hexanedioic acid bis(2-ethylhexyl) ester	2398

RT: Retention time; RI: Retention index.

provide specific percentages, hence we have refrained from citing such articles. The molecular structures of isothiocyanates extracted from various *Capparis* species are illustrated in Figure 5.

Isothiocyanates, which originate from glucosinolate precursors in cruciferous plants, are recognized as some of the most potent chemoprophylactic agents. Numerous studies affirm that both natural and synthetic isothiocyanates possess anticarcinogenic properties, as they not only diminish the activation of carcinogens but also augment their detoxification [44-48]. Moreover, they demonstrate antitumor capabilities, influencing a myriad of pathways such as apoptosis, MAPK

Table 5 Production of main isothiocyanates in essential oils of the genus *Capparis* collected in different world regions

Species, tissues	Collected place	1	2	4	5	6	7	8	12	13	17	Ref.
<i>C. flexuosa</i> , leaves	Brazil				11.2						79.3	[26]
<i>C. spinosa</i> , leaves and flower buds	Croatia	92.1			0.4		0.3					[11,27]
<i>C. spinosa</i> , leaves	Jordan	25.6		28.9								[28]
<i>C. cartilaginea</i> , leaves	Jordan	31.8	2.5	18.2		5.4						[28]
<i>C. spinosa</i> var. <i>aegyptiaca</i>	Egypt	24.7		12.4	3.2							[29]
<i>C. cartilaginea</i> , leaves	Egypt				65.0				29.9			[30]
<i>C. deserti</i> , leaves	Egypt				68.7				20.0			[30]
<i>C. spinosa</i> , fruits	Iran			13.7	10.6					15.6		[31]
<i>C. cartilaginea</i> , yellow fruits	Israel			48.7		1.4	49.4	0.1				This study
<i>C. cartilaginea</i> , green fruits	Israel		0.1	46.7		2.6	49.8	0.3				This study
<i>C. cartilaginea</i> , seeds	Israel			28.1		0.2	6.8					This study
<i>C. cartilaginea</i> , jam	Israel			1.2		0.1	3.3					This study
<i>C. spinosa</i> , leaves	Syria	25.6		28.9		16.6		2.2				[32]
<i>C. ovata</i> , buds	Turkey	4.5	1.5		0.1	0.2						[33]
<i>C. ovata</i> , leaves	Turkey	20.0	1.6		0.5	0.3						[33]
<i>C. cartilaginea</i> , leaves	Yemen			69.4	26.9	3.3						[34]

signaling, oxidative stress, and cell cycle progression[47-51].

The process through which natural isothiocyanates are formed *via* the hydrolysis of glucosinolates, facilitated by the enzyme β -thioglucosidase (known as myrosinase), is depicted in Figure 6. This biosynthetic mechanism is well-established, with isothiocyanates being identified in both plants and fungi[52-54]. Utilizing the PASS computer program, we computed the activity of natural isothiocyanates extracted from plants within the *Capparis* genus. The ensuing data is outlined in Table 7. As the table reveals, the primary properties pertaining to biological activity encompass apoptosis agonist, chemoprotective, chemosensitizer, and antineoplastic functions.

Benzyl isothiocyanate (9) has been extracted from *Capparis spinosa* (*C. spinosa*) components. Traditionally, fresh parts of this plant, particularly the flower buds, have been consumed as accompaniments to olives, cheese, and nuts. This plant stands out as one of the most cherished aromatic varieties native to the Mediterranean region. The fermentation of different parts of *C. spinosa* not only renders the capers consumable but also shapes their distinct taste, along with their organoleptic and nutritional attributes[54]. The biological activity of benzyl isothiocyanate is depicted in a 3D graph, as illustrated in Figures 7 and 8A.

Table 6 Production of main isothiocyanates in essential oils of the genus *Capparis* collected in different world regions

Species, tissues	Collected place	1	4	5	6	Ref.
<i>C. decidua</i> , leaf	Pakistan		11.0	6.3		[34]
<i>C. spinosa</i> , leaves	Spain	87.2		0.1	0.8	[35]
<i>C. spinosa</i> , stems	Spain	86.6		0.1	0.4	[35]
<i>C. spinosa</i> , flower buds	Spain	65.3				[35]
<i>C. spinosa</i> , aerial parts	Saudi Arabia	31.6		1.1		[36]
<i>C. cartilaginea</i> , leaves	Kenya	31.8			3.2	[37]

Advanced ovarian cancer cannot be cured by surgery alone; chemotherapy is vital for its treatment. While isothiocyanates have been shown to inhibit carcinogen-induced tumorigenesis in animal models, their therapeutic potential in advanced ovarian cancer remains unexplored. Kalkunte *et al*[55] demonstrated that benzyl isothiocyanate, commonly found in cruciferous vegetables like broccoli, cabbage, and watercress, suppresses the proliferation of advanced ovarian cancer cells and triggers apoptosis. Preliminary studies indicate its potential in both preventing and treating various cancers. Given this evidence, more research is essential to confirm its efficacy in humans and to advance its potential as a prophylactic or therapeutic agent, maximizing therapeutic outcomes while minimizing toxicity in cancer treatments[47].

In our study, we examined the volatile components of yellow and green fruits from the scrambling shrub *C. cartilaginea*. Additionally, we delved into the composition of seeds and jam derived from *C. cartilaginea* using GC/MS analysis. We detected isothiocyanates in all plant samples studied. This research presents a comprehensive overview of isothiocyanates identified in the *Capparis* genus, gathered from various global regions. Through the PASS program, we ascertained the biological activities of these isothiocyanates. Our findings revealed that these compounds are promising apoptosis agonists with potential as potent antitumor agents. Furthermore, we identified additional biological activities. The insights provided in this study hold substantial practical relevance and could pave the way for medical applications. The term “chemoprotective” refers to the properties of a substance that helps protect cells and tissues from the toxic effects of chemicals or against the DNA damage that can lead to cancer. In other words, chemoprotective agents help prevent or reduce the risk of chemically induced diseases, including various forms of cancer. Chemoprotective properties can arise from a variety of mechanisms: (1) Antioxidant activity: Many chemoprotective agents can neutralize free radicals, reducing oxidative stress, which can cause DNA damage and potentially lead to cancer; (2) Detoxification: Certain substances can enhance the body’s detoxification processes, helping to remove or neutralize potential carcinogens before they can cause harm; (3) Enhancement of DNA repair: Some agents can boost the mechanisms that repair damaged DNA; (4) Inhibition of carcinogen activation: Some chemicals need to be activated in the body to become carcinogenic. Chemoprotective agents can inhibit the enzymes responsible for this activation; (5) Suppression of carcinogen binding to DNA: By preventing carcinogens from binding to DNA, chemoprotective agents can reduce the risk of mutations that might lead to cancer; and (6) Inhibition of tumor growth: Some agents can slow or stop the growth of tumors by affecting cell cycle progression, inducing apoptosis (programmed cell death) or suppressing the blood supply to tumors (anti-angiogenesis).

Natural foods, especially fruits, vegetables, and spices, are rich sources of chemoprotective compounds. Examples include the isothiocyanates from cruciferous vegetables, polyphenols from green tea, curcumin from turmeric, and resveratrol from grapes, among many others. In the context of cancer, chemoprotection can also refer to strategies or agents used to protect normal tissues from the harmful side effects of chemotherapy while allowing the drugs to act on cancer cells.

Ethyl-(2), allyl-(14), and 3-methyl-3-butenyl-isothiocyanates (17) exhibited a pronounced apoptosis agonist activity, with confidence levels exceeding 95%. The associated 3D graph (Figure 8B) visually represents their activities. Another visual representation can be observed in Figure 8C, where three specific isothiocyanates stand out due to their robust anti-*Helicobacter pylori* activity, which exhibits over 80% confidence. Among these, anticancer properties are the most prominent.

Furthermore, isothiocyanates labeled as 26, 27, and 28 provide compelling data, as illustrated in Figure 8D. Not only do these compounds demonstrate potent apoptosis agonist activity, surpassing 93% confidence, but they also show promise in treating periodontitis with a confidence level exceeding 70%.

“Anti-*Helicobacter pylori* activity” refers to the ability of a substance to inhibit or eradicate *Helicobacter pylori* bacteria. *Helicobacter pylori* is a type of bacteria that can infect the stomach and is known to be a main cause of peptic ulcers, and its persistent infection has also been linked to stomach cancer. Therefore, substances with anti-*Helicobacter pylori* activity may help in preventing or treating these conditions.

Substances with anti-*Helicobacter pylori* activity might function through various mechanisms, such as: (1) Inhibiting the growth or reproduction of the bacteria; (2) Killing the bacteria directly; and (3) Disrupting the mechanisms by which the bacteria cause disease (for instance, by neutralizing toxins produced by the bacteria).

Anti-*Helicobacter pylori* activity can be exhibited by antibiotics, as well as various other natural and synthetic compounds, and is an area of interest in pharmacology and medicinal chemistry due to the importance of managing infections by this bacterium. Research into substances with anti-*Helicobacter pylori* activity may yield new treatments for infections and possibly for preventing stomach ulcers and cancer.

Table 7 Predicted biological activity of isothiocyanates derived from essential oils of the genus *Capparis*

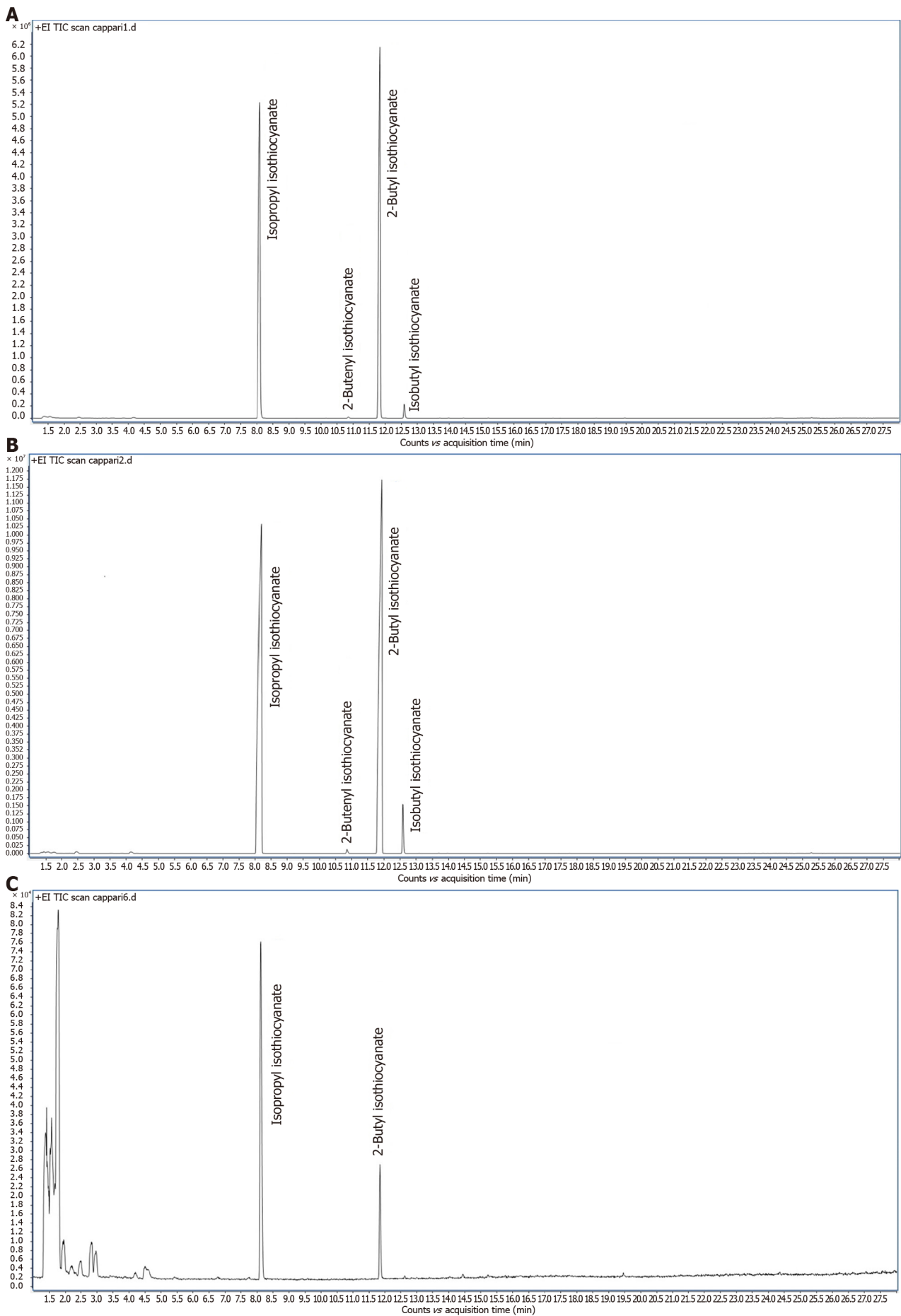
No	Anticancer properties. Pa ¹	Anti-infectives properties. Pa ¹
1	Apoptosis agonist (0.963)	Anti-schistosomal (0.759)
	Chemoprotective (0.871)	Antiviral (arbovirus) (0.638)
	Antineoplastic (0.794)	Anti-seborrheic (0.614)
2	Apoptosis agonist (0.965)	Anti- <i>Helicobacter pylori</i> (0.853)
	Chemoprotective (0.890)	Anti-seborrheic (0.749)
	Chemosensitizer (0.798)	Anti-schistosomal (0.711)
	Antineoplastic (0.789)	Antiparasitic (0.537)
3	Apoptosis agonist (0.956)	Anti- <i>Helicobacter pylori</i> (0.816)
	Chemoprotective (0.866)	Anti-seborrheic (0.690)
	Chemosensitizer (0.779)	Antiviral (arbovirus) (0.635)
	Antineoplastic (0.743)	Anti-schistosomal (0.612)
4	Apoptosis agonist (0.914)	Anti- <i>Helicobacter pylori</i> (0.720)
	Chemoprotective (0.819)	Anti-schistosomal (0.687)
	Chemosensitizer (0.726)	Anti-seborrheic (0.684)
5	Apoptosis agonist (0.956)	Anti- <i>Helicobacter pylori</i> (0.816)
	Chemoprotective (0.858)	Antiviral (arbovirus) (0.690)
	Chemosensitizer (0.778)	Anti-schistosomal (0.594)
	Antineoplastic (0.751)	Antiparasitic (0.570)
6	Apoptosis agonist (0.951)	Anti- <i>Helicobacter pylori</i> (0.804)
	Chemoprotective (0.850)	Anti-seborrheic (0.731)
	Chemosensitizer (0.765)	Anti-schistosomal (0.665)
	Antineoplastic (0.720)	Antiparasitic (0.524)
7	Apoptosis agonist (0.867)	Antiviral (arbovirus) (0.654)
	Chemoprotective (0.782)	Anti-seborrheic (0.650)
	Chemosensitizer (0.694)	Anti- <i>Helicobacter pylori</i> (0.624)
8	Apoptosis agonist (0.955)	Anti- <i>Helicobacter pylori</i> (0.822)
	Chemoprotective (0.839)	Antiparasitic (0.680)
	Antineoplastic (0.833)	Anti-helminthic (0.632)
	Chemosensitizer (0.791)	Antifungal (0.568)
9	Apoptosis agonist (0.965)	Anti- <i>Helicobacter pylori</i> (0.629)
	Antineoplastic (0.825)	Anti-schistosomal (0.598)
	Chemoprotective (0.782)	
	Chemosensitizer (0.696)	
10	Apoptosis agonist (0.933)	Anti- <i>Helicobacter pylori</i> (0.739)
	Chemoprotective (0.847)	
	Antineoplastic (0.728)	
	Chemosensitizer (0.722)	
11	Apoptosis agonist (0.884)	Anti- <i>Helicobacter pylori</i> (0.679)
	Chemoprotective (0.812)	
	Antineoplastic (0.714)	
	Chemosensitizer (0.661)	

12	Apoptosis agonist (0.956)	Anti- <i>Helicobacter pylori</i> (0.703)
	Chemoprotective (0.825)	
	Chemosensitizer (0.741)	
	Antineoplastic (0.740)	
	Antineoplastic (genitourinary cancer) (0.581)	
13	Apoptosis agonist (0.856)	Anti- <i>Helicobacter pylori</i> (0.703)
	Chemoprotective (0.825)	
	Chemosensitizer (0.741)	
	Antineoplastic (0.740)	
	Antineoplastic (genitourinary cancer) (0.581)	
14	Apoptosis agonist (0.959)	Anti- <i>Helicobacter pylori</i> (0.792)
	Chemoprotective (0.867)	Antiparasitic (0.609)
	Chemosensitizer (0.787)	Anti-helminthic (0.581)
	Antineoplastic (0.775)	Antis-chistosomal (0.574)
15	Apoptosis agonist (0.923)	Anti- <i>Helicobacter pylori</i> (0.659)
	Chemoprotective (0.817)	Antifungal (0.658)
	Antineoplastic (0.771)	Antiparasitic (0.560)
	Chemosensitizer (0.728)	
16	Apoptosis agonist (0.919)	Anti- <i>Helicobacter pylori</i> (0.752)
	Chemoprotective (0.821)	Antiviral (arbovirus) (0.730)
	Antineoplastic (0.751)	Antifungal (0.678)
	Chemosensitizer (0.747)	Antiparasitic (0.672)
17	Apoptosis agonist (0.953)	Anti- <i>Helicobacter pylori</i> (0.753)
	Chemoprotective (0.830)	Antifungal (0.533)
	Antineoplastic (0.781)	
	Chemosensitizer (0.754)	
18	Antineoplastic (myeloid leukemia) (0.805)	Anti-eczematic (0.606)
	Chemosensitizer (0.742)	
19	Apoptosis agonist (0.952)	Anti- <i>Helicobacter pylori</i> (0.769)
	Chemoprotective (0.847)	Anti-eczematic (0.610)
	Chemosensitizer (0.764)	Antifungal (0.575)
	Antineoplastic (0.753)	Anti-schistosomal (0.502)
20	Apoptosis agonist (0.955)	Anti- <i>Helicobacter pylori</i> (0.901)
	Chemoprotective (0.911)	Anti-ulcerative (0.611)
	Antineoplastic (0.781)	
	Chemosensitizer (0.694)	
	Anticarcinogenic (0.573)	
	Chemopreventive (0.559)	
21	Apoptosis agonist (0.851)	Anti- <i>Helicobacter pylori</i> (0.691)
	Chemoprotective (0.839)	
	Chemosensitizer (0.747)	
	Antineoplastic (0.733)	
	Antineoplastic (genitourinary cancer) (0.627)	

22	Apoptosis agonist (0.951)	Anti-seborrheic (0.775)
	Antineoplastic (0.820)	Antifungal (0.543)
	Chemoprotective (0.752)	Anti- <i>Helicobacter pylori</i> (0.542)
	Chemosensitizer (0.673)	
	Preneoplastic conditions treatment (0.559)	
23	Apoptosis agonist (0.929)	Anti- <i>Helicobacter pylori</i> (0.780)
	Chemoprotective (0.832)	Antiviral (arbovirus) (0.626)
	Chemosensitizer (0.771)	Antiparasitic (0.589)
	Antineoplastic (0.762)	Anti-helminthic (0.559)
	Preneoplastic conditions treatment (0.515)	
24	Apoptosis agonist (0.932)	Anti- <i>Helicobacter pylori</i> (0.736)
	Chemoprotective (0.819)	Anti-schistosomal (0.579)
	Chemosensitizer (0.742)	Antifungal (0.550)
	Antineoplastic (0.675)	Antiviral (arbovirus) (0.531)
	Preneoplastic conditions treatment (0.541)	Antiparasitic (0.529)
25	Apoptosis agonist (0.930)	Anti- <i>Helicobacter pylori</i> (0.715)
	Chemoprotective (0.828)	Antiviral (arbovirus) (0.639)
	Antineoplastic (0.792)	Antifungal (0.620)
	Chemosensitizer (0.754)	
26	Apoptosis agonist (0.938)	Periodontitis treatment (0.752)
	Chemoprotective (0.827)	Anti- <i>Helicobacter pylori</i> (0.739)
	Antineoplastic (0.740)	Antifungal (0.622)
	Chemosensitizer (0.736)	Antiviral (arbovirus) (0.599)
	Preneoplastic conditions treatment (0.593)	
27	Apoptosis agonist (0.937)	Periodontitis treatment (0.727)
	Chemoprotective (0.820)	Anti- <i>Helicobacter pylori</i> (0.718)
	Antineoplastic (0.742)	Antifungal (0.644)
	Chemosensitizer (0.728)	Antiviral (arbovirus) (0.563)
	Preneoplastic conditions treatment (0.563)	
28	Apoptosis agonist (0.934)	Periodontitis treatment (0.751)
	Chemoprotective (0.823)	Anti- <i>Helicobacter pylori</i> (0.733)
	Antineoplastic (0.740)	Antifungal (0.639)
	Chemosensitizer (0.732)	Antiviral (arbovirus) (0.621)
	Preneoplastic conditions treatment (0.613)	

¹Only activities with Pa > 0.5 are shown.

Periodontitis refers to a serious gum infection that damages the soft tissue and destroys the bone that supports your teeth. It can lead to tooth loss or worse, if not treated. Periodontitis is common but largely preventable. It is usually the result of poor oral hygiene. Key points about periodontitis include: (1) Cause: It is primarily caused by bacteria that adhere to and grow on the tooth's surfaces, along with an aggressive immune response against these bacteria; (2) Symptoms: Red or swollen gums, tender or bleeding gums, painful chewing, loose teeth, sensitive teeth, bad breath that does not go away, and receding gums or longer appearing teeth; (3) Risk factors: Periodontitis can be influenced by several factors including poor oral hygiene, tobacco use, diabetes, age, genetics, certain medications, and other conditions like decreased immunity; (4) Complications: If left untreated, periodontitis can result in tooth loss. It can also increase the risk of stroke, heart attack, and other health problems; and (5) Treatment: Treatment usually involves good dental hygiene practices, scaling, and root planning (deep cleaning) to remove the plaque and tartar, and in more severe cases, surgical treatments. Regular dental checkups and good oral hygiene can help prevent periodontal disease.



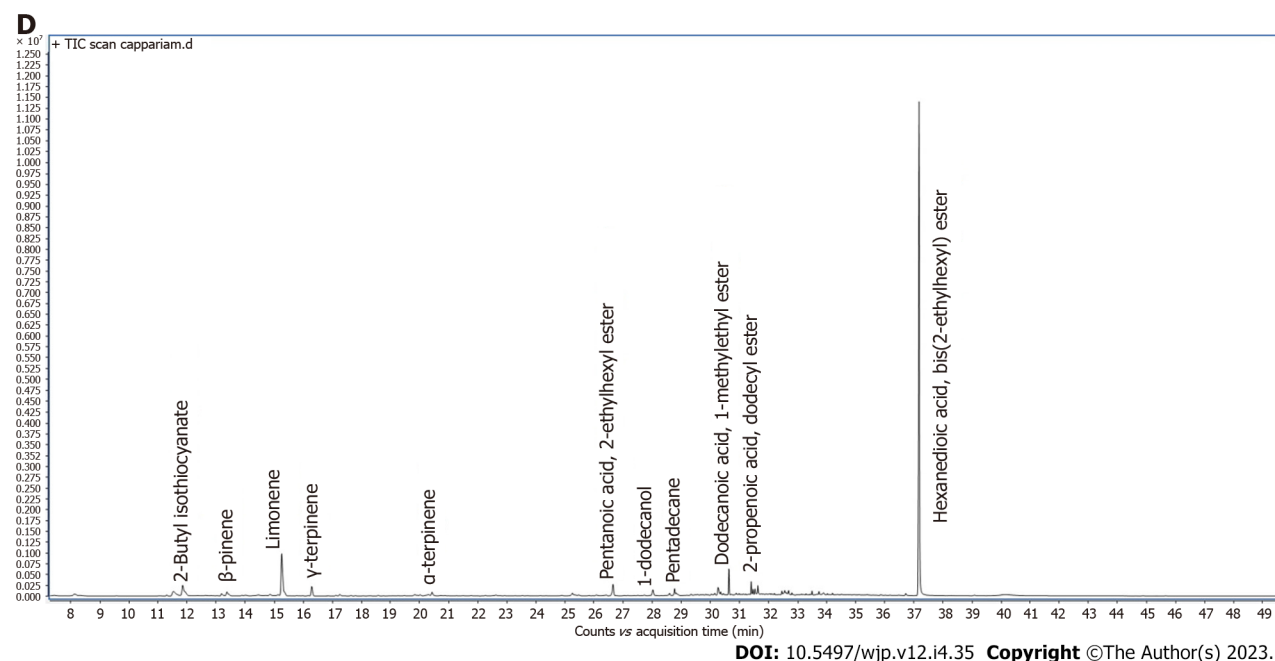


Figure 2 Gas chromatography-mass spectrometry chromatogram of compounds which were identified from yellow and green fruits, seeds, and jam of *Capparis cartilaginea*. A: Yellow fruits; B: Green fruits; C: Seeds; D: Jam.



Figure 3 Seeds of the scrambling shrub *Capparis cartilaginea*.

CONCLUSION

In our study, we examined the volatile components of yellow and green fruits from the scrambling shrub *C. cartilaginea*. Additionally, we delved into the composition of seeds and jam derived from *C. cartilaginea* using GC/MS analysis. We detected isothiocyanates in all plant samples studied. This research presents a comprehensive overview of isothiocyanates identified in the *Capparis* genus, gathered from various global regions. Through the PASS program, we ascertained the biological activities of these isothiocyanates. Our findings revealed that these compounds are promising apoptosis agonists with potential as potent antitumor agents. Furthermore, we identified additional biological activities. The insights provided in this study hold substantial practical relevance and could pave the way for medical applications.

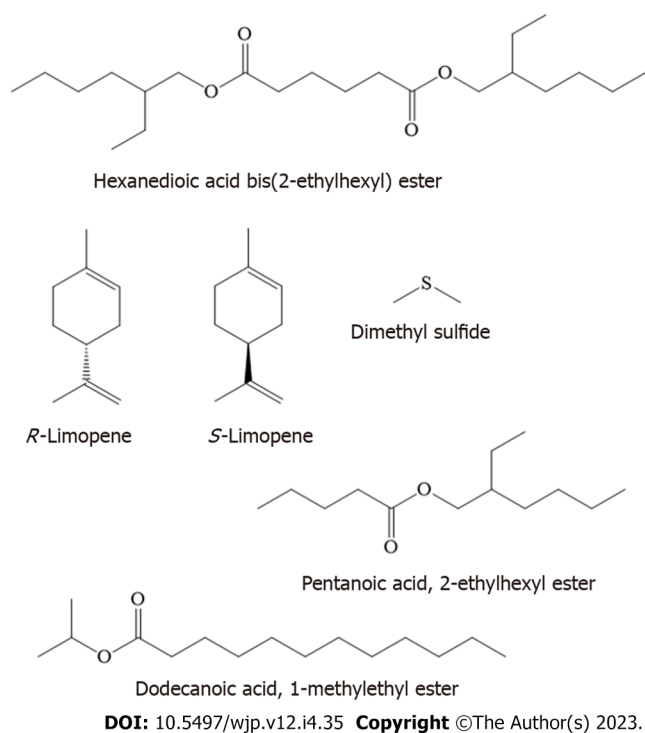


Figure 4 Major metabolites that have been identified in *Capparis cartilaginea* jam.

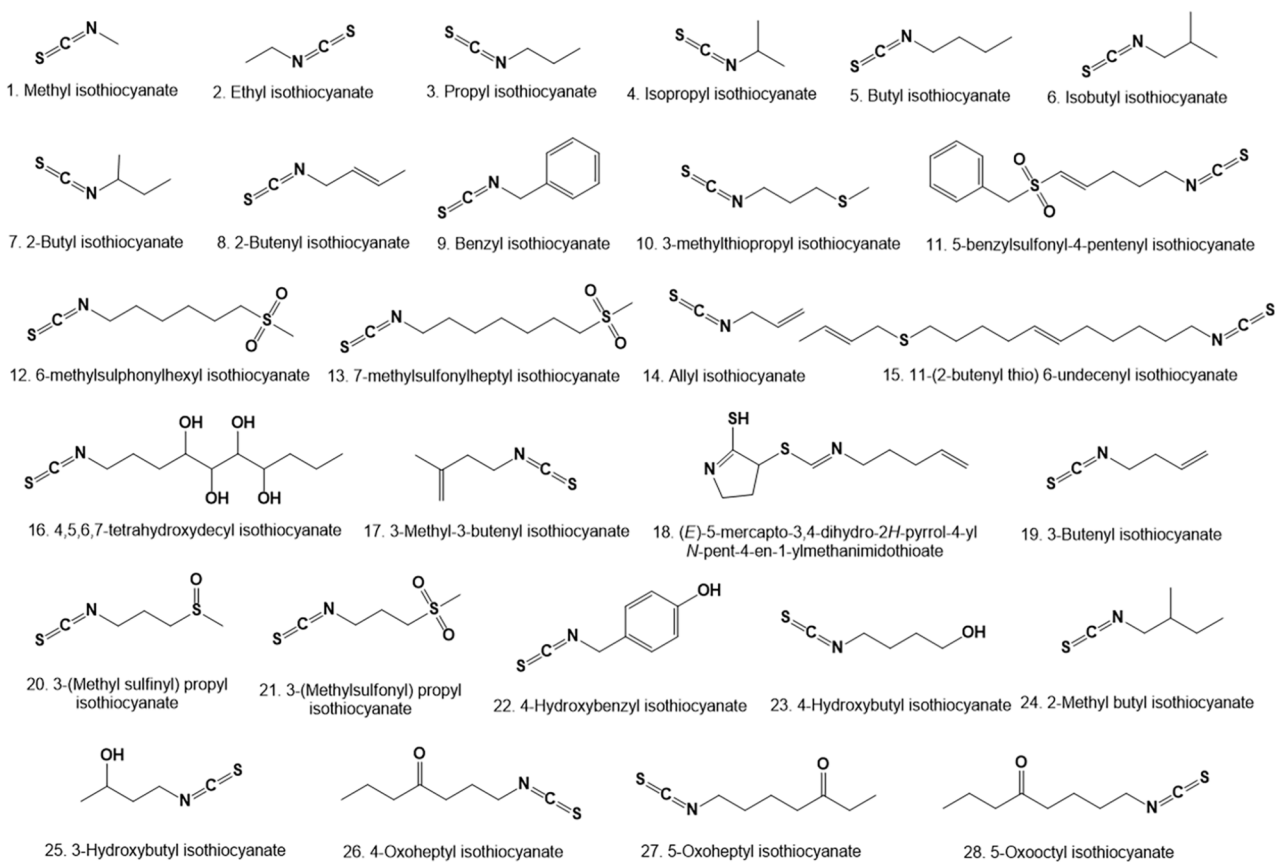
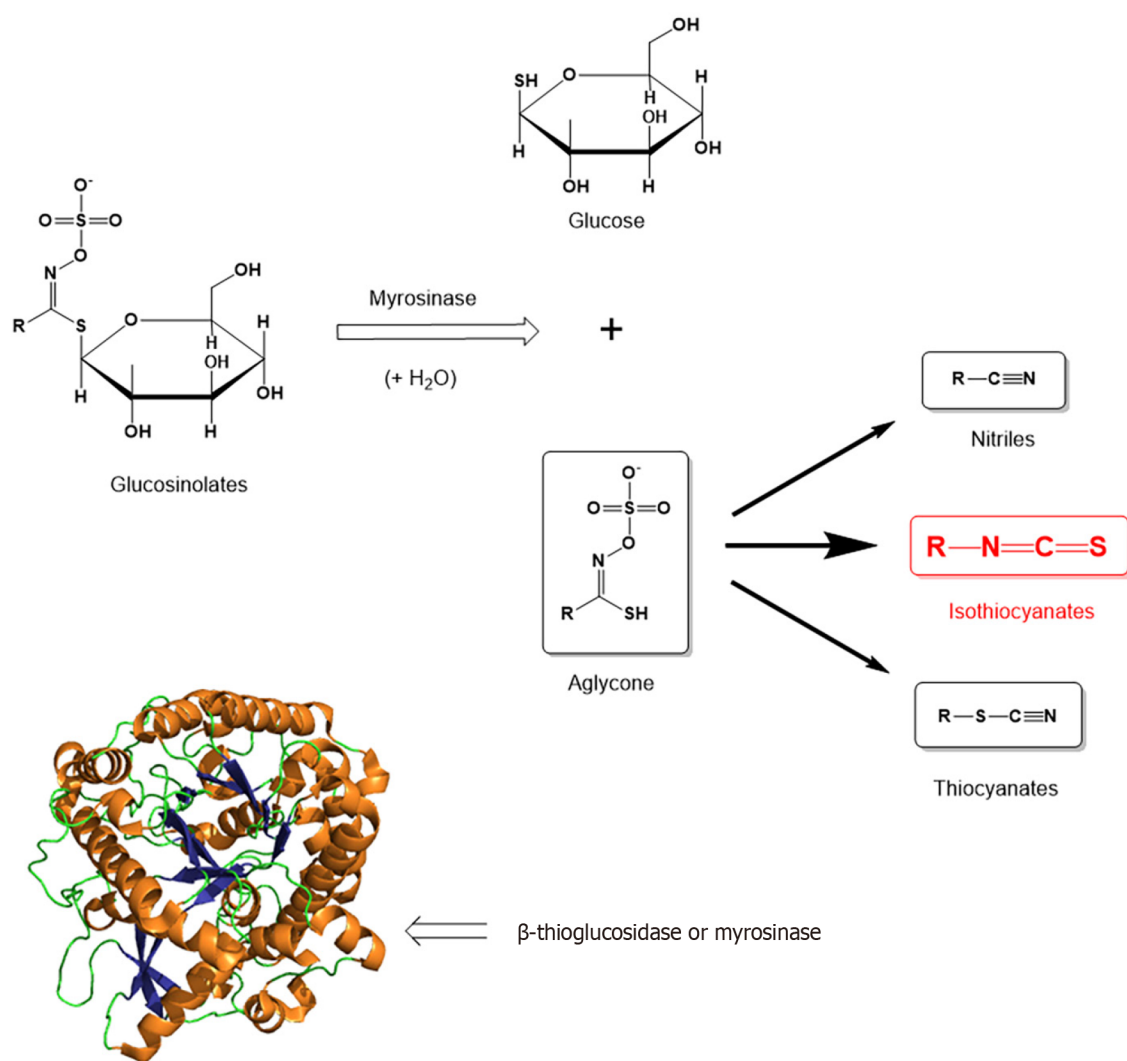
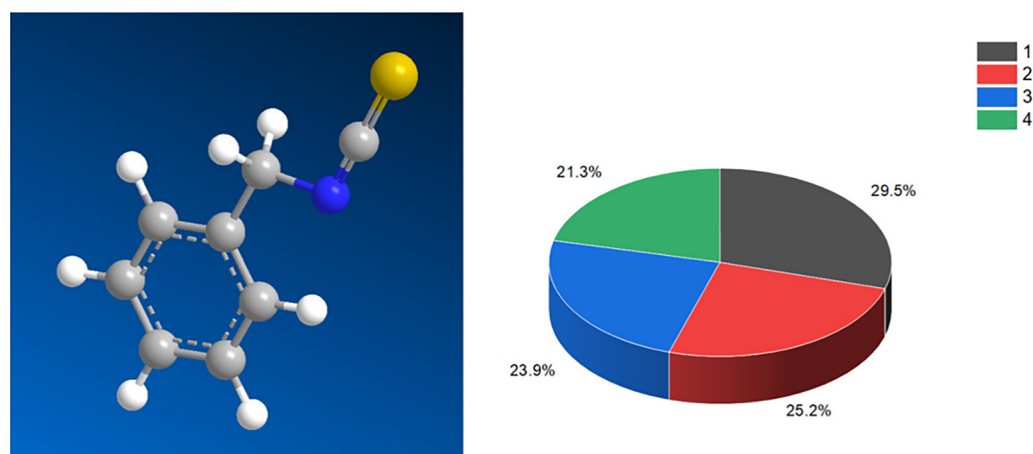


Figure 5 Isothiocyanates found in plant extracts of the genus *Capparis*. These compounds were identified by gas chromatography-mass spectrometry and other physical-chemical methods[26-43].



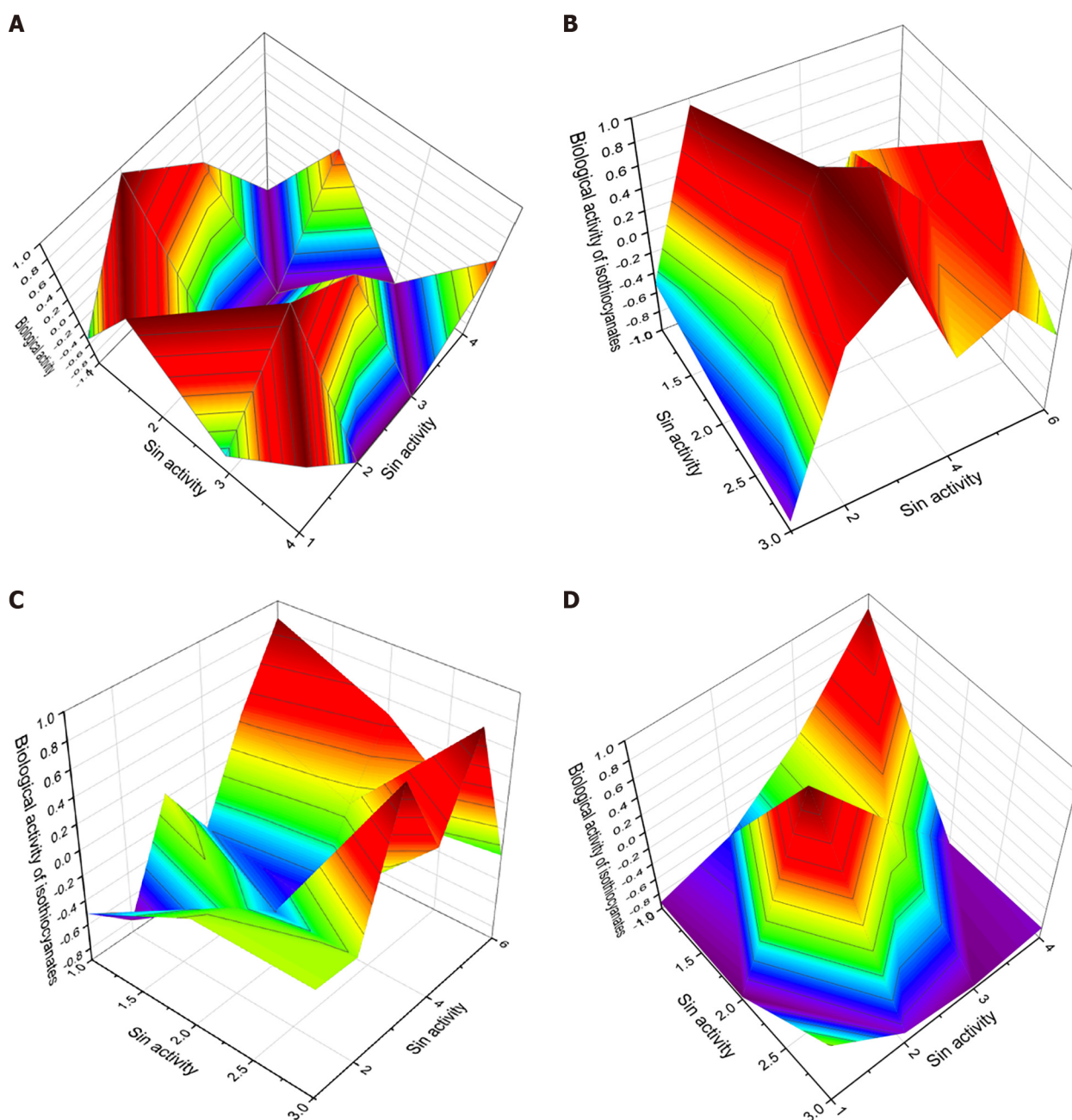
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Figure 6 Myrosinase (or β -thioglucosidase) which catalyzes the hydrolysis of glucosinolates to isothiocyanates, thiocyanates, nitriles, and other metabolites.



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Figure 7 3D model (left) and percentage distribution of the dominant biological activity on the example benzyl isothiocyanate (9), which has a wide range of anticancer properties. Where activities are indicated under the numbers: (1) Apoptosis agonist (29.5%); (2) Antineoplastic (25%); (3) Chemoprotective (23.9%); and (4) Chemosensitizer (21.3%). The nitrogen atom is highlighted in blue, and sulfur atom is highlighted in brown.



DOI: 10.5497/wjp.v12.i4.35 Copyright ©The Author(s) 2023.

Figure 8 3D graphs. A: 3D graph shows a wide range of biological activities and predicted pharmacological activities of benzyl isothiocyanate (9). This compound is characterized as an agonist of apoptosis. In addition, it exhibits antitumor properties and is an inhibitor of the development of the Gram-negative microaerophilic helical bacterium *Helicobacter pylori* (*H. pylori*). The *H. pylori* infection is known to be an important public health problem worldwide, with a prevalence of 45% to 84%. The *H. pylori* bacteria enter the digestive tract and can cause ulcers in the lining of the stomach or in the upper part of the small intestine, and patients can develop chronic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, stomach cancer, or peptic ulcer disease. Amoxicillin is commonly used to treat this infection, and it appears that isothiocyanates may be a potential drug for *H. pylori* infection; B: 3D graph shows the predicted and calculated biological activity of isothiocyanates (compound numbers: 2, 14, and 17) showing the highest degree of confidence. All presented natural isothiocyanates have a dominant activity as an apoptosis agonist with a confidence of more than 96%. The second activity that characterizes these isothiocyanates is chemoprotective; C: 3D graph shows the predicted and calculated anti-*H. pylori* activity of isothiocyanates (compound numbers: 5, 8, and 20) showing the highest degree of confidence, more than 82.2%; D: 3D graph shows the predicted and calculated activity of isothiocyanates against periodontitis (compound numbers: 26, 27, and 28) showing the highest degree of confidence, more than 73%.

ARTICLE HIGHLIGHTS

Research background

In the realm of medicinal chemistry, isothiocyanates are characterized by the $-N=C=S$ functional group, which results from substituting the oxygen atom in the isocyanate group with sulfur. These compounds are predominantly found in plants and arise from the enzymatic conversion of metabolites, specifically glucosinolates. Notably, numerous plant-derived isothiocyanates have demonstrated anticarcinogenic properties. Their mechanism of action involves inhibiting

the activation of carcinogens and bolstering their detoxification processes.

Research motivation

Our motivation to undertake this study stemmed from the noticeable lack of extensive literature regarding isothiocyanates in food sources. While some health research has touched upon the use of isothiocyanates, comprehensive investigations into their potential benefits remain limited. Consequently, we embarked on an in-depth *in silico* study of isothiocyanates to assess their preliminary therapeutic properties.

Research objectives

To investigate the composition of fruits, seeds, and jam derived from the scrambling shrub *Capparis cartilaginea* (*C. cartilaginea*) utilizing gas chromatography-mass spectrometry (GC-MS) analysis, and to conduct an *in silico* examination of the biological activity associated with the isolated isothiocyanates.

Research methods

For our investigation, we employed the following methods: GC/MS analysis: This technique allowed us to accurately identify and quantify the volatile components present in the samples from the scrambling shrub *C. cartilaginea*; PASS computer program: We utilized the PASS software, which boasts a comprehensive database of over one million natural and synthetic compounds, paired with more than 10000 documented biological activities. As per data from its official website, this German-developed program is a popular tool among the scientific community, with over 26000 researchers from 34 different countries using it on an annual basis.

Research results

Our investigation revealed that isothiocyanates exhibit a significant anticancer potential. Additionally, these compounds display other potential biological activities, including antiviral, antibacterial, and antifungal properties.

Research conclusions

The findings from our investigation are promising. We identified the presence of isothiocyanates in jams, seeds, and fruits, which demonstrated potential anti-cancer properties. Nevertheless, further *in vitro* and *in vivo* studies are essential to validate these preliminary results.

Research perspectives

Moving forward, the intention is to conduct more in-depth GC/MS and PASS *in silico* analyses on individual isothiocyanates extracted from jams, seeds, and fruits of the *Capparis* genus. This will provide a clearer understanding of the properties and potential therapeutic applications of these compounds.

FOOTNOTES

Co-corresponding authors: Lumír Hanuš and Valery M Dembitsky.

Author contributions: Hanuš L carried out the extraction and analysis of volatile components of yellow and green fruits, seeds, and jam from the scrambling shrub *Capparis cartilaginea*; Naor T grew the material and provided it for analysis; Gloriovova T determined the biological activity of volatile components; Dembitsky VM prepared the article for publication and also wrote and reviewed this article; and all authors read and approved the final version of the manuscript. In addition, the co-corresponding authors contributed equally to the accompanying manuscript, such as describing the methods, their application to the analysis, and writing the discussion.

Institutional review board statement: The study was conducted *in silico* and did not include humans or animals, so a statement from the Institutional Review Board was not necessary.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The study was conducted only in a computational environment and the data and three-dimensional structures used are available in public online databases.

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S-Editor: Wang JJ

L-Editor: Wang TQ

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