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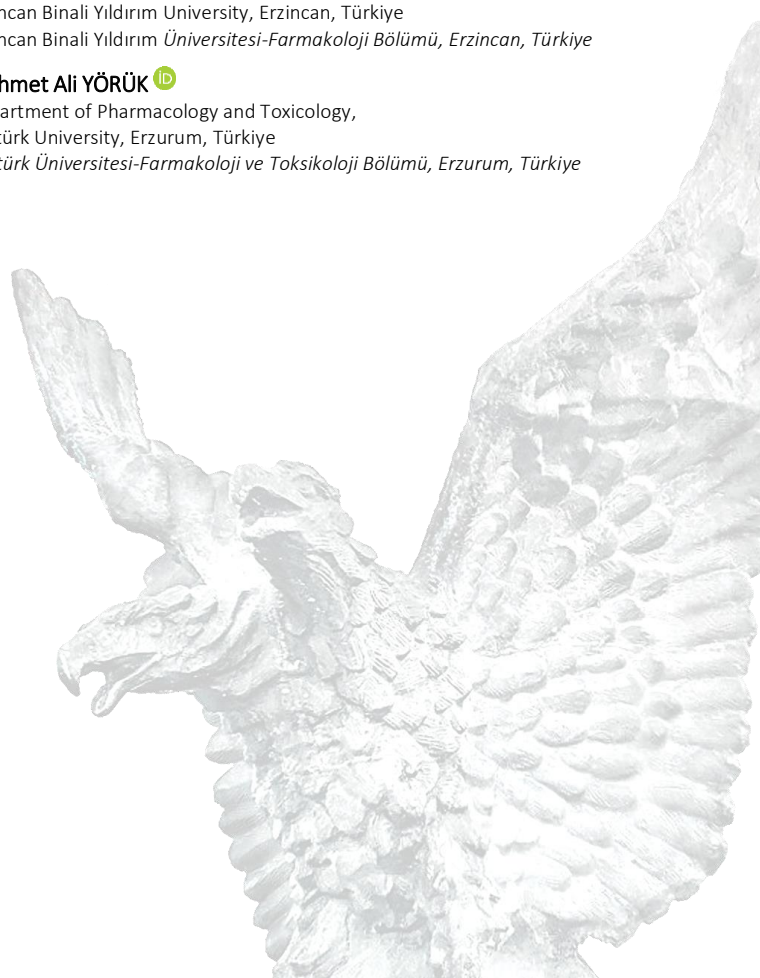

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ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

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Unveiling Ecopharmacology: Insights Into Knowledge, Attitude, and Practices Among Medical and Nursing Students in Tertiary Care Teaching Hospital of District Almora, Uttarakhand

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ABSTRACT

Objective: Ecopharmacology, a field emerging at the intersection of pharmacology and environmental science, focuses on the impact of pharmaceuticals on ecosystems and public health. Given the increasing recognition of environmental issues associated with pharmaceutical waste, understanding the knowledge, attitudes, and practices (KAP) of health-care students toward Ecopharmacology is crucial. This study aims to assess KAP toward ecopharmacology among medical and nursing student at a tertiary care centre.
Methods: This descriptive and cross-sectional study employed a pre-validated self-administered structured online questionnaire conducted between December 2024 and January 2025. The questionnaire consisted of 22 questions to assess the knowledge, attitude, and practices of medical and nursing students regarding ecopharmacology. The data were subsequently analysed and presented as percentages.
Results: Out of 468 consenting participants 453 (291 Bachelor of Medicine, Bachelor of Surgery [MBBS] students and 162 nursing students) satisfactorily completed the questionnaire. The mean age was found to be 21.31±1.76 years. The result showed that in response to knowledge both MBBS and nursing students had good knowledge of certain aspects of ecopharmacology while knew less about drug-take back system. Regarding attitude they were empathetic towards the issue and worried about safe disposal of the drugs. The majority (89.3% medical and 97.5% nursing students) of participants agreed regarding the need for guidelines for ecologically safe disposal of drugs by people, whereas 77.3% of medical students reported discarding of excess medicines in the household trash.
Conclusion: The present study highlights a significant level of awareness and concern regarding pharmaceutical waste management among medical and nursing students.

Keywords: Ecopharmacology, Environmental Pharmacology, KAP study, Medicine Disposal, Unused Medicines

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Introduction

The presence of medicinal products in the environment is a globally emerging issue. Ecopharmacology is the science concerned with the entry of chemicals or drugs into the environment through any route and at any concentration disturbing the balance of ecology (ecosystem), as a consequence (Rahman et al., 2007). Ecopharmacology is also referred to as environmental pharmacology or ecopharmaco-stewardship. The unused or expired medicines can pose a hazard to public safety and to the environment, if they are not safely disposed of, as many drugs lead double lives: one within the body of animal/humans and one in the environment (Ling et al., 2024).

These drugs pass out of the system either as metabolites or unchanged through excretion. These drugs are found in traces in environment, mostly in water (Joss et al., 2006).

Drugs enter the environment due to various factors, including consumption, improper disposal, and pharmaceutical company effluents. There are pharmaceutical waste management guidelines, such as Good Manufacturing Practice (GMP) and Food and Drug Administration (FDA) regulations, which apply to manufacturing units, pharmacists, and consumers (Kadam et al., 2016).

Though the consumption phase is considered to be the biggest contributor to the emissions of medicinal products into the environment, through excretions and incorrect disposal of unused medicines through sinks and toilets, there is unawareness of correct disposal methods. Cocaine, oral contraceptives, carbamazepine and iodine contrast media are some of the examples of drugs found in traces in environment mostly in water (Zuccato et al., 2005, Kolpin et al., 2002). Cocaine has been detected in the Po River in Italy, while antidepressants, antiepileptics, and lipid-regulating agents (statins) have been found in the Niagara River (Castiglioni et al., 2004, Heberer, 2002). These drugs then have impact on human beings and animals through environment. The example of this is study from Pakistan which revealed that due to the use of diclofenac in treatment of livestock and consumption of their dead bodies by vultures led to the kidney failures of these vultures (Oaks et al.,2004). Similarly in India also the population of vultures declined so drastically that they were declared endangered species. Because of this Government of India banned diclofenac in India for veterinary use (Taggart et al., 2007).

To ascertain the potential environmental impact of improperly disposed medications in Almora district, it is necessary to determine the number of unused medications that are not currently returned to a pharmacy and disposed of via the landfill or water systems. Educating medical and nursing students about pharmaceutical waste management could serve as a significant catalyst for instigating change. To optimize the clinical proficiency medical and nursing students and cultivate environmentally conscious health-care practitioners, the integration of ecopharmacology appears to be a crucial prerequisite. To achieve this objective, a prior assessment of medical and nursing students' knowledge, attitudes, and practices (KAP) concerning drug disposal is imperative. Hence, this study was planned with the objective to assess KAP among medical and nursing students toward ecopharmacology. This will help in formulating and executing strategies to empower future Doctors and Nursing staff in assuming a more substantial responsibility for the safe disposal of pharmaceutical waste, as well as for educating the public about this matter.

Methods

Study design

A descriptive cross-sectional study was conducted from December 2023 to January 2024 using a pre-validated self-administered structured online questionnaire. The questionnaire was formulated in English, adapted, and modified from previous studies (Khanavkar et al., 2024, Advani et al., 2019). A pilot study involving a subset of medical and nursing students was conducted to assess the reliability and validity of the questionnaire. The findings from the pilot study were not included in the final analysis. Medical and nursing students of either gender from tertiary care hospitals were included in the study. Participation in the study was voluntary. Informed consent was obtained from the participants electronically before administering the questionnaire. Approval was obtained from the Institutional Ethical Committee of Soban Singh Jeena Government Institute of Medical Science and Research Almora. Date of approval 12/07/2024. IEC approval number – 560/GMC/ IEC/ Reg. No. 518/ IEC/ R- 12-05-2024.

Sample size and sampling method

A non-probability sampling technique (convenience method) was employed to reach a representative population within our institute.

Study instrument

The questionnaire consisted of two sections. Section one was about respondent's personal information including gender, age and education. Section two of the questionnaire included questions related to participants knowledge, attitude and practices regarding ecopharmacology. The questionnaire consisted of 22 questions. After explaining the study objectives and obtaining consent, Participants were given 20–25 minutes to complete the questionnaire, which was provided in English only. Data was collected using Google Forms, recorded in Microsoft Office Excel 2007, and analysed using appropriate analytical software.

Results

In this study, out of 468 consenting participants 453 (291 MBBS students and 162 nursing students) satisfactorily completed the questionnaire and were included for evaluation. The mean age was found to be 21.31±1.76 years

(Table 1). Among the 453 participants, the majority were female. The response of participants regarding knowledge of "Ecopharmacology" was as summarised in table 2. The result showed that in response to knowledge both MBBS and nursing students had good knowledge about few aspects of ecopharmacology while knew less about drug-take back system. Table 3 depicted attitude of the respondents and found out that they were empathetic towards the issue and worried about safe disposal of the drugs. Table 4 depicted the practices of respondents towards ecopharmacology and found that MBBS students were involved in not stocking drugs than nursing students.

Table 1. Socio - demographic of the respondents (n= 453)

Variables		No. (%)
Age	<21 years	149
	≥21 years	304
Mean ± SD	21.31±1.76	
Gender	Male	155 (34.2)
	Female	298 (65.8)
MBBS Students	Batch 2021	96 (21.2)
	Batch 2022	98 (21.6)
	Batch 2023	97 (21.4)
Nursing Students	2nd Year	40 (8.8)
	3rd Year	39 (8.6)
	4th Year	42 (9.3)
	Intern	41 (9.1)

Table 2. Knowledge of Respondent regarding Ecopharmacology (n= 453)

Questions	Yes (No.%)		No (No.%)	
	MBBS Students	Nursing Students	MBBS Students	Nursing Students
1. Have you heard of the term Ecopharmacology?	194 (66.7)	131 (80.9)	97 (33.3)	31 (19.1)
2. Do you feel drugs that you consumed can cause environmental (soil and water) pollution?	254 (87.3)	156 (96.3)	37 (12.7)	06 (3.7)
3. Do you think environmental pollution caused by drugs can be a reason for antibiotic resistance?	241 (82.8)	146 (90.1)	50 (17.2)	16 (9.9)
4. Can the expired/unused medicines which are not properly disposed, cause hazard to public safety?	281 (96.6)	160 (98.8)	10 (3.4)	02 (1.2)
5. Do you know about the drug-take back system?	91 (31.3)	70 (43.2)	200 (68.7)	92 (56.8)
6. Have you heard the terms "Green Chemistry" and "Green Pharmacy"?	207 (71.1)	66 (40.7)	84 (28.9)	96 (59.3)
7. Do you think use of Ayurvedic, Homeopathic, and Veterinary drugs can affect environment?	197 (67.7)	87 (53.7)	94 (32.3)	75 (46.3)

Table 3. Attitude of Respondent regarding Ecopharmacology (n= 453)

Questions	Yes (No.%)		No (No.%)	
	MBBS Students	Nursing Students	MBBS Students	Nursing Students
1. Have you been taught the proper disposal of medication in college or have you received information from healthcare professionals?	202 (69.4)	129 (79.6)	89 (30.6)	33 (20.4)
2. Has it ever bothered you, what to do with excess medicine?	206 (70.8)	132 (81.5)	85 (29.2)	30 (18.5)
3. Do you think there should be guidelines for public for ecologically safe disposal of drug?	282 (96.9)	156 (96.3)	09 (3.1)	06 (3.7)
4. Do you think there should be safe medicine disposal location?	281 (96.6)	155 (95.7)	10 (3.4)	07 (4.3)
5. Do you think manufactures and pharmacist should have drug take back system?	274 (94.2)	150 (92.6)	17 (5.8)	12 (7.4)
6. Do you feel its your responsibility to protect environment from pharmaceutical waste?	284 (97.6)	160 (98.8)	07 (2.4)	02 (1.2)
7. Would you like to participate in activities like campaign for this issue?	260 (89.3)	158 (97.5)	31 (10.7)	04 (2.5)
8. Do you think Ecopharmacology knowledge will be relevant in your medical practice?	280 (96.2)	158 (97.5)	11 (3.8)	04 (2.5)

Table 4. Practice of Respondent regarding Ecopharmacology (n= 453)

Questions		MBBS Students	Nursing Students
1. Do you buy drugs in bulk for your family members?	Yes (Yes.%)	79 (27.1)	18 (11.1)
	No (No.%)	212 (72.9)	144 (88.9)
2.Do you store leftover unused /unwanted medications in your house?	Yes (Yes.%)	151 (51.9)	39 (24.1)
	No (No.%)	140 (48.1)	123 (75.9)
3. Do you remove drug from its container before throwing in the garbage?	Yes (Yes.%)	86 (29.6)	129 (79.6)
	No (No.%)	205 (70.4)	33 (20.4)
4. Do you pour leftover syrup/lotion from the bottle in the wash basin?	Yes (Yes.%)	67 (23)	95 (58.6)
	No (No.%)	224 (77)	67 (41.4)
5. Are you willing to dispose of unused medications through proper collection programs?	Yes (Yes.%)	278 (95.5)	150 (92.6)
	No (No.%)	13 (4.5)	12 (7.4)
6. How do you dispose of your unused medication?	Down the toilet	11 (3.8)	12 (7.4)
	Down the sink	09 (3.1)	50 (30.9)
	Returned to pharmacy	46 (15.8)	21 (13)
	With household rubbish	225 (77.3)	79 (48.8)
7. Why do you keep unused medications?	Do not want to waste them	29 (10)	06 (3.7)
	In case they needed later	165 (56.7)	85 (52.5)
	To keep a stockpile in case of emergency	97 (33.3)	71 (43.8)

Discussion

Ecopharmacovigilance highlights the importance of controlling the sources of pharmaceutical waste to prevent environmental contamination. Improper disposal of medications poses significant risks, leading to environmental pollution, disruption of the food chain, and harm to various life forms, including microorganisms (Kümmerer et al., 2006). The present study provides insights into the knowledge, attitudes, and practices (KAP) of medical and nursing students regarding ecopharmacology and medication disposal. The high response rate of 96.75% from the 468 participating HCPs indicates a strong interest and engagement in the topic among the study population. In the present study, in terms of knowledge, the majority of participants (66.7% medical and 80.9% nursing students) correctly defined the term 'ecopharmacology,' indicating a solid understanding of the concept among the respondents. This finding is similar to the studies conducted by khanavkar where 84% of correctly defined the term 'ecopharmacology (Khanavkar et al., 2024).

Most of our participants (87.3% medical and 96.3% nursing students) felt that the drugs they consume could cause environmental pollution and were aware of the hazardous environmental and health impact of improper disposal of unused and expired medicines. Studies of drug disposal practices in university students from Kabul and general population from Serbia have also reported of awareness on this issue (Bashaar et al., 2017, Seehusem et al., 2006). In present study 82.8% medical and 90.1% nursing participants agreed that environmental pollution by drugs can be reason for antibiotic resistance. This finding is similar to the studies conducted by Advani and Jadhao (Advani et al., 2019) and Bhadoriya and Wadagbalkar (Bhadoriya et al., 2014), where 88% of medical students expressed the belief that environmental pollution caused by drugs could contribute to antibiotic resistance. In present study 96.6% medical and 98.8% nursing students agreed that the expired/unused medicines which are not properly disposed, pose hazards to public safety. This finding is similar to the studies conducted by Advani and Jadhao (Advani et al., 2019).

The present study also revealed some gaps among nursing students in knowledge, particularly regarding specific terms such as "Green Chemistry" and "Green Pharmacy," with approximately half of the nursing participants (40.7%) familiar with these concepts. However, the majority of medical students (71.1%) have recognized this term. This result is consistent with other studies conducted in Saudi Arabia (Alhomoud et al., 2021) and Bangladesh (Shakib et al., 2022). Green chemistry is an area of chemistry and chemical engineering focused on the design of products and processes that minimize or eliminate the use and generation of hazardous substances (Srivastava et al., 2022). Whereas, Green pharmacy is a concept that has evolved as a response to the increased insight into the harmful effects of pharmaceuticals. Included in the concept are measures to minimize the environmental footprint during all stages of the medicine's life cycle, from research and development via prudent prescribing and dispensing to safe disposal (Daughton et al., 2011).

However, the majority of participants (67.7% medical students) have recognized the potential environmental impact of Ayurvedic, Homeopathic, and Veterinary drugs. This percentage are lesser with the findings of a study conducted by khanavkar where 75% of medical students had recognized the potential environmental impact of Ayurvedic, Homeopathic, and Veterinary (Khanavkar et al., 2024). But these observations are consistent with the findings of a study conducted in Kosovo (Shuleta-Qehaja et al., 2022).In this study, we observed that majority of participants (69.4% medical and 79.6% nursing students) had been taught about proper disposal of pharmaceuticals in medical school or by health professionals, which is much higher than the study conducted by Gubae et al. (2023) where only (27%) had been taught about proper disposal practices (Gubae et al., 2023).

The Majority of respondents (70.8% medical and 81.5% nursing students) expressed concern about the presence of unused or unwanted medications in the home and supported the implementation of policies to safely dispose of such medications (96.9% medical and 96.3% nursing students). Almost all the participants (96.6% medical and 95.7% nursing students) were willing to dispose of medications in specific locations or in an appropriate place. These observations are Consistent with study conducted among health care students in Gaza (Tabash et al., 2016). Almost all the participants (97.6% medical and 98.8% nursing students) agree that it's their responsibility toward environmental protection. Also, the majority (89.3% medical and 97.5% nursing students) expressed willingness to engage in proper medication disposal programs. These observations are consistent with the findings of a study conducted by Advani and Jadhao (2019), Gubae et al. (2023) and Khanavkar (2024).

The present study identified positive response of

participants regarding medication management and disposal practices. Very few (27.1% medical and 11.1% nursing students) of participants disclosed buying drugs in bulk for family members, while 58.6% nursing students admitted to pouring leftover syrup/lotion into the wash basin. Similar result was reported in studies from Kuwait, UK and USA (Tong et al. (2011), Avahussain et al. (2007). But these findings are significantly higher than those reported in the study conducted by Advani and Jadhao (2019). Present study participants were willing about the disposal of the excess medicines at home but were unaware of safe disposal methods. There are world health organization (WHO) guidelines for 'safe disposal of unwanted pharmaceuticals' (WHO guideline, 1999). This is a finding reflected in our study where majority (89.3% medical and 97.5% nursing students) of participants agreed regarding the need for guidelines for ecologically safe disposal of drugs by people. Responses from 77.3% of medical students showed that they throw away the excess medicines in the household trash. This is similar to findings of the study an Asian study in which most of the respondents (234/301; 77.7%) were throwing the expired medicine in household trash while 21.3% of the respondents returned unused and expired pharmaceuticals to medical stores. Returning expired and unused medicines to medical stores is community practice in the USA (23%) and UK (22%) (Bashaar et al., 2017; Azad et al., 2012; Kuspis, 1996). Whereas in present study, only few (15.8% medical and 13% nursing students) of the participants have done this. In this study the most common reasons that unused medications were keeping for future use (56.7% medical and 52.5% nursing students). These observations are consistent with other studies conducted among healthcare students (Auta et al., 2012; Khalid Labu et al., 2013).

Limitations of the study

Because this is a cross-sectional study, causal relationships cannot be established. Results are based on self-report and therefore may be biased by recall. Our decision to include only health care students may have affected the external validity of the results. However, this study is the first to assess medication disposal practices from the perspective of knowledge and attitudes toward ecopharmacology.

Conclusion

From the questionnaire it was observed that the knowledge of medical personnel and student about ecopharmacology and problems associated with it was good. Attitude of medical personnel and students was also very empathetic, but this is not transforming into practices. Gaps exist in knowledge and practices, therefore robust, safe and cost-effective pharmaceutical waste management program supported with media campaign is needed.

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Ethical approval: Approval from Institutional Ethical Committee of Government Medical College Almora was taken. Date of approval 12/07/2024. IEC approval number – 560/GMC/ IEC/ Reg. No. 518/ IEC/ R- 12-05-2024.
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Low-Dose Hydrogen Sulfide Ameliorates Cisplatin-Induced Hepatotoxicity in Rats

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ABSTRACT

Objective: Cisplatin (CIS) is non-specific to the cell cycle, has a cytotoxic effect, and is used in many cancers. The side effects of CIS, such as hepatotoxicity, seriously limit its clinical use. This experimental study aims to prevent hepatotoxicity, one of the clinical side effects that cisplatin may cause. At the same time, Hydrogen sulfide (H2S) will be applied prophylactically and therapeutically and its effect levels will be compared. This study aims to contribute to the literature by preventing dose restriction due to the hepatotoxicity side effect of cisplatin, which plays an important role in cancer treatment, by prophylactic application of H2S. Our study investigated the protective and therapeutic efficacy of sodium hydrosulfide (NaHS, a donor of H2S), which activates the antioxidant system on CIS-induced hepatotoxicity.

Method: Control (Vehicle), CIS (7.5 mg/kg CIS), H2S+CIS (10 µmol/kg NaHS+7.5 mg/kg CIS), CIS+H2S (7.5 mg/kg CIS+10 µmol/kg NaHS) groups were formed by using 35 rats in the study. At the end of the study, blood and liver tissue was taken, and histopathological and biochemical analyzes were performed.

Results: It was determined that sinusoidal dilatation and congestion increased significantly in the CIS group and decreased in the H2S+CIS and CIS+H2S groups. Likewise, glycogen loss occurred in the CIS group, and a significant improvement was observed in the H2S+CIS group. In addition, significant deterioration was detected in malondialdehyde, catalase, glutathione, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase values in the CIS group compared to the control group. In contrast, significant improvements were observed in both the H2S+CIS and CIS+H2S groups compared to the CIS group.

Conclusion: Both protective and therapeutic beneficial effects of H2S in CIS-induced hepatotoxicity were demonstrated by histopathological and biochemical analyses.

Keywords: Cisplatin, Hepatotoxicity, Hydrogen Sulfide, Oxidative Stress, Rat

Introduction

The liver has many essential roles, including the metabolism of nutrients, the synthesis of glucose and lipids, immunity, vitamin storage, and the detoxification of drugs and xenobiotics (Trefts et al., 2017; Koroglu et al., 2021). Drug-induced liver injury (DILI) is still known as the most common cause of acute liver failure in the West. The prevalence of DILI varies depending on geographic location, but the estimated annual incidence worldwide is between 1.3 and 19.1 per 100.000 exposed person (Garcia-Cortes et al., 2020). DILI can be severe and life-threatening in humans, resulting in significant morbidity/mortality and high healthcare costs (Amirana & Babby, 2015).

Cisplatin (CIS) is a widely used antineoplastic drug and is still used in the treatment of breast, cervical, esophageal, bladder, small cell lung, osteosarcoma, squamous cell carcinoma, and testicular cancer and has an important place in treatment protocols (Gao et

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al., 2021; Man et al., 2020). Although CIS is widely used, it has significant side effects. Its important side effects include nephrotoxicity, hepatotoxicity, cardiotoxicity, and its clinical use is limited due to these side effects (Neamatallah et al., 2018; Hassan et al., 2020; Darwish et al., 2017; Hwang et al., 2020). There are not many known pathways for CIS-induced hepatotoxicity, which began with an overabundance of reactive oxygen species (ROS) that caused oxidative stress, inflammation, DNA damage, and liver death (Abd Rashid et al., 2021). CIS causes oxidative/nitrosative stress-mediated damage and disruption of cellular function in many subcellular structures, especially lipids, proteins, and DNA in the cellular structure (Peres & da Cunha, 2013). In many experimental models, it has been suggested that CIS causes toxicity by disrupting the oxidative stress balance and increasing inflammation and apoptosis (Bentli et al., 2013; Lu & Cederbaum, 2006; Omar et al., 2016). As a result of the studies, it was determined that free radicals and (ROS) increased in the toxicity models induced by CIS, and it was shown that lipid peroxidation increased and glutathione levels decreased (Pratibha et al., 2006). However, the mechanisms of CIS-induced hepatotoxicity are not fully understood (Fathy et al., 2022).

Hepatotoxicity caused by CIS, which has widespread clinical use, necessitates discovering preventive and therapeutic agents (Al-Malki & Sayed, 2014). Hydrogen sulfide (H2S) is an endogenous gasotransmitter that can add a hydropersulfide moiety (-SSH) to the cysteine residue in target proteins and stimulate catalytic activity (Paul & Snyder, 2018). H2S, previously known as toxic, is a gas that acts as a regulator in many physiological processes as a result of recent studies (Feng et al., 2020; Yuan et al., 2017). It is known that H2S, in particular, plays a vital role in regulating hepatic physiology and pathology. Studies have shown that H2S has many protective and therapeutic (antioxidant, anti-inflammatory, antiapoptotic, cytoprotective) effects (Calvert et al., 2010). H2S, widely recognized and known as an antioxidant, has been shown to scavenge ROS directly (Yi et al., 2019). Although there are many sources of intracellular ROS, the most important source is the NADPH oxidase 4 (Nox4) based NADH/NADPH oxidase system (Crosas-Molist & Fabregat, 2015). Studies have also shown that it regulates signalling pathways in physiological functions such as kinase regulation and maintenance of mitochondrial ATP production (Cohen et al., 2013; Yan et al., 2013; Ye et al., 2020; Fu et al., 2012). Several studies have demonstrated the beneficial effects of H2S on endotoxemia, acetaminophen, microplastics, and nickel-induced liver damage (Fu et al., 2012; Li et al., 2021). NaHS (a donor of H2S) has been extensively applied in clinical trials to predict the biological effects of H2S (Li et al., 2015; Kimura, 2014).

Although many studies are showing the efficacy of H2S, its preventive and therapeutic efficacy in CIS-induced hepatotoxicity is unknown, yet (Azarbarz et al., 2020; Karimi et al., 2017; Ibrahim et al., 2022; Kwon et al., 2019; Tu et al., 2016).

This study aims to create a hepatotoxicity model in rats with cisplatin, a widely used antineoplastic drug, and to reveal the prophylactic and therapeutic effects of H2S, which has antioxidant, anti-inflammatory, antiapoptotic, cytoprotective, etc. effects.

Methods

Animals

Thirty-five Sprague Dawley rats (3–4 months, 200–250 g) were purchased from Inonu University Laboratory Animal Research Center. Rats were housed on a light and dark (12:12) cycle in a room with controlled temperature (21±3◦C) and humidity (60±3%). Rats were fed standard chow and normal water, and rats were given ad libitum access. During the experiment, animal care and all experimental procedures were performed following the National Institutes of Health Animal Research Guidelines and ARRIVE guideline 2.0 (Percie du Sert et al., 2020).

The protocol of the study was approved by the Animal Research Ethics Committee, Inonu University, Malatya, Faculty of Medicine (Protocol: 2015/A-84- 22.10.2015). The simple randomization technique formed the experimental groups. Also, all procedures performed during the experiment and evaluating the results obtained were studied blindly.

Chemicals

The main component, CIS (Cisplatin DBL 100 mg/100 ml, Orna, Istanbul, Turkey) and NaHS (CAS number: 16721-80-5, Sigma-Aldrich, St Louis, MO) was purchased. Ketamine hydrochloride (Ketalar®) and Xylazine HCl (Alfamine®) were purchased from erse Medikal, Istanbul, Turkey.

Experimental Design

For the experiment, 35 male Sprague Dawley rats were simply randomly divided into four groups and the experimental model is described in Figure 1. In our study, we used NaHS, the donor of H2S.

1. Control group (n=8): Rats were given 0.5 mL of NaCl 0.9 % solution via per-oral (p.o.) for 14 days (Hashmi et al., 2021).

2. CIS group (n=9): A single dose of 7.5 mg/kg CIS was administered to the rats via intraperitoneal (i.p.) (Wang et al., 2022).

3. H2S+CIS group (n=9): Rats were given a dose of 10 µmol/kg NaHS for 12 days via p.o. and a single dose of 7.5 mg/kg CIS intraperitoneally via i.p. on the 13th day (Otunctemur et al., 2014).

4. CIS+H2S group (n=9): After a single of 7.5 mg/kg CIS to rats via i.p. administration and the next 12 days, 10 µmol/kg NaHS was administered via i.p (Pan et al., 2009).

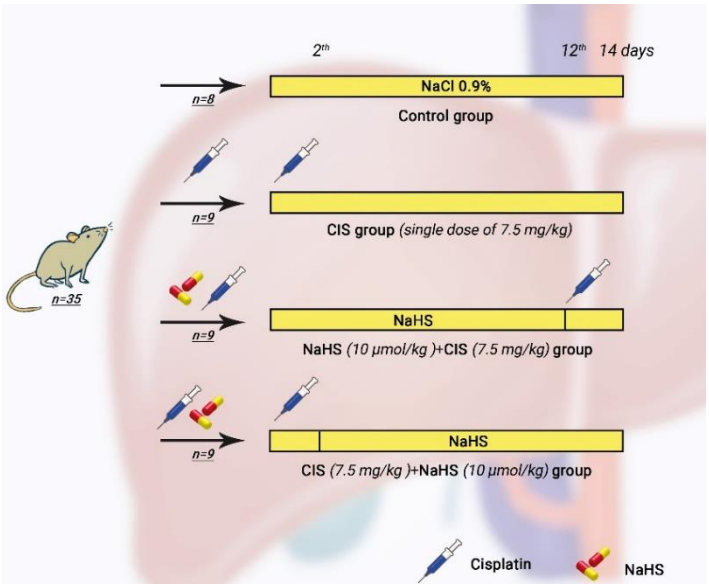

Figure 1. Schematic representation of the experimental design.

The body weights of the rats were weighed at the beginning and end of the study, as well as their liver weights at the end of the study. At the end of the experiment, all rats were sacrificed after ketamine and xylazine (75 mg/kg and 5 mg/kg, intraperitoneally) mixture administration. Immediately before the scarification procedure, blood samples were collected from the inferior vena cava for biochemical analysis. Hepatectomy was carried out after blood collection, and part of the liver sample was fixated with formalin for histopathological examination. The remaining tissues were stored at -70 ◦C for biochemical analysis. In addition, blood samples were taken into tubes without anticoagulant for the determination of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)]. Biochemical examinations [malonyldialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px)] and histopathological (light microscopy) were performed on liver tissue at the end of the experiment.

Biochemical Analysis

Liver The liver samples were homogenized (IKA ultra turrax T 25 basic) in cold phosphate buffer (pH 7.4). The homogenates were centrifuged (10000xg for 20 min at +4˚C), and the enzyme (MDA, SOD, CAT, GSH, and GSH-Px) levels determination was made in the supernatant part. The blood samples taken from rats were centrifuged at 2000xg for 10 min at +4˚C, and the obtained serum samples were used to measure the levels of AST, ALT, and LDH. The serum samples were frozen at -70˚C until assayed. The samples were taken to +4˚C one day before the biochemical analysis for correct analysis and thawing. AST, ALT, and LDH parameters were studied at Turgut Ozal Medical Center Laboratories (Abbott Architect c16000), Inonu University, Malatya, Turkey.

MDA, an indicator of lipid peroxidation, was studied according to the method of Uchiyama and Mihara (Mihara and Uchiyama, 1978). The rat liver sample was homogenized on ice for 1 minute at 15000 rpm to form 10% homogenate in 1.15% KCl solution. In the spectrophotometer, the absorbance of the supernatant was read at 535 nm. The result was shown as nmol/g tissue.

Tissue SOD activity was measured according to the method of Sun et al. (Sun et al., 1988). After adding 3 to 5 chloroform/ethanol mixture to the supernatants, all samples were centrifuged for 20 minutes at 5000 rpm at +4 degrees. Subsequently spectrophotometric evaluation of the samples at 560 nm was performed. Enzyme activity was given as U/g protein.

Tissue CAT activity was measured according to Luck's (De Bruijn, 1981) method. The rat liver sample was homogenized on ice for 1 min at 15000 rpm to form 10% homogenate. The absorbance at 240 nm was read immediately after the supernatant was added to the sample tubes. Enzyme activity was given as K/mg protein.

GSH was determined according to the method of Ellman (Ellman, 1959). The rat kidney sample was homogenized on ice to form 10% homogenate at 15000 rpm for 1-2 min. Then, the homogenate was centrifuged at 3000 rpm at +4 degrees for 15 min. Then, after adding TCA solution to the supernatant, it was mixed homogeneously and

centrifugation was repeated. After the protocol was completed, the samples were read at 410 nm in the spectrophotometer. GSH levels were presented as nmol/g tissue.

The GSH-Px activity was also determined according to the method of Paglia and Valentine (Paglia and Valentine, 1967) and measured by monitoring its oxidation at 340 nm. GSH-Px levels were presented as U/g protein).

Histopathological Analysis

At the end of the experiment, the liver tissue was fixed in 10% formaldehyde. After the tissue follow-up procedures, 4-5 µm thick sections were taken from the paraffin blocks prepared. Hematoxylin-eosin (H-E) staining methods were applied to the areas for general histological evaluations, and periodic acid schiff (PAS) staining methods were applied to determine glycogen loss. Sections using the H-E staining method, sinusoidal dilatation, sinusoidal congestion, and necrosis were examined. Damage according to its severity, ten randomly selected areas were evaluated by scoring 0 (no injury), 1 (light injury), 2 (moderate injury), and 3 (serious injury). In the PAS staining method, ten randomly selected areas were examined, and according to the prevalence of glycogen loss, 0; normal, 1; less than 25% change, 2; the difference between 25-50%, 3; It was evaluated by scoring with more than 50% change (Bilgic et al., 2018). Leica DFC-280 microscope was used for histopathological analysis and Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) was used for imaging analysis.

Data Analysis

Statistical power analysis was performed to determine the required power and sample sizes. Considering type I error (alpha) 0.05, power (1-beta) 0.8 and effect size 0.92 for AST levels, it was determined that the minimum sample size required to detect a significant difference should be at least 8 in each group (Arslan et al., 2018). The normality of the distribution was verified using the Kolmogorov-Smirnov test. Mann-Whitney U test was used for intergroup comparisons in histopathological analyzes. Post-hoc Tukey's test was applied after ANOVA to compare groups in biochemical analyses. Statistical evaluation of histopathological and biochemical analyzes was performed using SPSS (IBM SPSS Corp., Armonk, NY, USA) for Windows version 25. $p < .05$ was accepted as the significance level.

Results

Mortality, Weight, and Liver-Body Weight Gain Ratio

Two rats in the CIS group and one rat in the CIS+ H2S group died due to toxicity caused by CIS during the drug administration period. At the end of the experiment, there was a statistically significant decrease in rat and liver weights in the CIS, H2S+CIS, and CIS+H2S groups compared to the control group ($p < .05$). Although there was no statistically significant difference in rat and liver weights in the H2S+CIS and CIS+H2S groups compared to the CIS group, an improvement tendency was detected. In contrast, no statistically significant difference was observed between the groups in terms of liver/rat weight ($p > .05$). Mortality, weight, and rate of increase in organ-body weight are presented in the Table 1.

Table 1. Descriptive statistics for liver, rat weight, and liver/rat weight ratios

Groups	Rat weight-Before (g)	Rat weight-After (g)	Liver weight (g)	Liver/rat weight ratio
Control (*n*=8)	308 (202-342)	316 (276-343)	10.57 (9.72-12.46)	.037 (.03-.043)
CIS (*n*=7)	255 (219-330)	207[a] (172-289)	7.49[a] (7.08-11)	.038 (.025-.051)
H_2S+CIS (*n*=9)	300 (268-355)	250[a] (190-300)	9.485[a] (6.17-11.3)	.039 (.025-.05)
CIS+H_2S (*n*=8)	325 (280-360)	253[a] (210-332)	8.605[a] (7.1-11.57)	.034 (.025-.055)
p	.05479	.00332	.01409	.913

g: Gram.

Biochemical Finding

Liver function tests

There was a significant increase in AST, ALT, and LDH levels in the CIS group when compared to the control group, and a significant decrease in the H2S+CIS group and CIS+H2S group compared to the CIS group ($p < .05$). Serum biochemistry results are presented in the Table 2.

Tissue biochemical findings

There was a significant increase in MDA level in the CIS group compared to the control group, and a significant decrease in the H2S+CIS group and the CIS+H2S group compared to the CIS group ($p < .05$). There was a significant decrease in CAT and GSH levels in the CIS group compared to the control group, and a significant increase in the H2S+CIS group and CIS+H2S group compared to the CIS group ($p < .05$). No significant difference was found between the groups in terms of SOD and GSH-Px levels ($p > .05$). Liver tissue biochemistry results are presented in the Table 3.

Table 2. Serum biochemical parameters.

Parameters	Groups			
	Control (*n*=8)	**CIS (*n*=7)**	**H_2S+CIS (*n*=9)**	**CIS+H_2S (*n*=8)**
AST (U/L)	163.38 ± 32.40	342.75 ± 96.65[a]	155.13 ± 68.98[b]	158.88 ± 52.83[b]
ALT (U/L)	78.00 ± 12.39	215.63 ± 160.17[a]	61.38 ± 24.10[b]	72.13 ± 20.38[b]
LDH (U/L)	730.38 ± 224.00	1560.63 ± 613.58[a]	737.13 ± 403.10[b]	657.88 ± 251.43[b]

[a]$p < .05$: Significant compared to the control group.

[b]$p < .05$: Significant compared to CIS group.

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase.

Table 3. Biochemical parameters of liver tissue**.**

Groups	Parameters				
	MDA (nmol/g tissue)	**SOD (U/g protein)**	**CAT (K/mg protein)**	**GSH (nmol/g tissue)**	**GSH-Px (U/g protein)**
Control (*n*=8)	141.75 ± 21.33	325.50 ± 206.20	2.77 ± 1.13	1226.75 ± 214.33	212.25 ± 111.58
CIS (*n*=7)	233.13 ± 40.90[a]	344.13 ± 62.77	0.89 ± 0.54[a]	788.88 ± 271.01[a]	241.38 ± 118.01
H_2S+CIS (*n*=9)	155.75 ± 30.51[b]	313.63 ± 101.36	2.66 ± 1.07[b]	1263.00 ± 287.6[b]	196.00 ± 97.98
CIS+H_2S (*n*=8)	145.50 ± 35.92[b]	341.63 ± 137.60	2.60 ± 1.24[b]	1267.88 ± 379.6[b]	219.38 ± 136.69

[a]$p < .05$: Significant compared to the control group.

[b]$p < .05$: Significant compared to CIS group.

MDA: Malonyldialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; GSH-Px: Glutathione peroxidase.

Histopathological Findings

The histological structure of the liver had a normal appearance in the control group. In sections stained with H-E, hepatocyte cords anastomoses around central veins and sinusoids between these cords were clearly observed (Figure 2-A). PAS (+) staining was observed in the cytoplasm of hepatocytes due to glycogen accumulation (Figure 3-A). It was observed that sinusoidal dilatation and congestion increased significantly in the CIS group (Figure 2-B and C). In addition, necrotic areas were observed around the central vein in some sections in the CIS group (Figure 2-B). In the sections where the PAS staining method was applied, it was observed that PAS (+) areas decreased due to glycogen loss, especially around the portal area in the CIS group (Figure 3-B). Sinusoidal dilatation and congestion were observed to be statistically significantly decreased in the H2S+CIS and CIS+H2S groups compared to the CIS group ($p < .05$) (Figure 2-D and E). On the other hand, it was observed that glycogen loss decreased statistically significantly in the H2S+CIS group compared to the CIS group ($p < .05$) (Figure 3-C), but it was observed that it continued in the CIS+H2S group similarly to the CIS group ($p > .05$) (Figure 3-D). There was no statistical difference between the CIS+H2S group and the H2S+CIS group in terms of sinusoidal dilatation, congestion and necrosis ($p > .05$). However, it was observed that glycogen loss was higher in the CIS+H2S group than in the H2S+CIS group ($p < .05$). Also, histopathological analysis scores on liver tissue are given in Table 4.

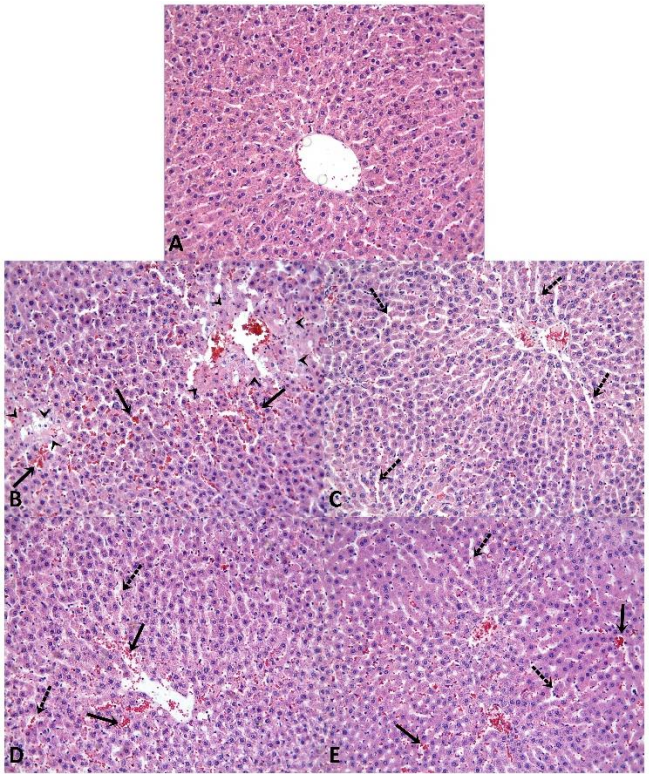

Figure 2. Liver tissue in the control group (A) has a normal histological appearance. Necrotic areas (arrowheads), sinusoidal congestion (arrows) and dilatation (dashed arrows) are observed around the central vein in the CIS group (B and C). It is observed that histological changes in the H2S+CIS (D) and CIS+H2S (E) groups are milder than the CIS group.

Table 4. Descriptive statistics for histopathological scores.

Parameters	Groups			
	Control (*n*=8)	**CIS (*n*=7)**	**H_2S+CIS (*n*=9)**	**CIS+H_2S (*n*=8)**
Sinusoidal Dilation	0.0 (0.0-1.0)	1.0 (0.0-3.0)[a]	1.0 (0.0-3.0)[b]	0.0 (0.0-2.0)[b]
Sinusoidal Congestion	0.0 (0.0-2.0)	1.0 (0.0-3.0)[a]	0.0 (0.0-2.0)[b]	0.0 (0.0-2.0)[b]
Necrosis	0.0 (0.0-0.0)	0.0 (0.0-3.0)[a]	0.0 (0.0-0.0)[b]	0.0 (0.0-0.0)[b]
Glycogen Loss	1.0 (0.0-1.0)	1.0 (0.0-3.0)[a]	0.0 (0.0-3.0)[b]	1.0 (0.0-3.0)[c]

[a] $p < .05$: Significant compared to the control group.

[b] $p < .05$: Significant compared to CIS group.

[c] $p < .05$: Significant compared to the H_2S+CIS

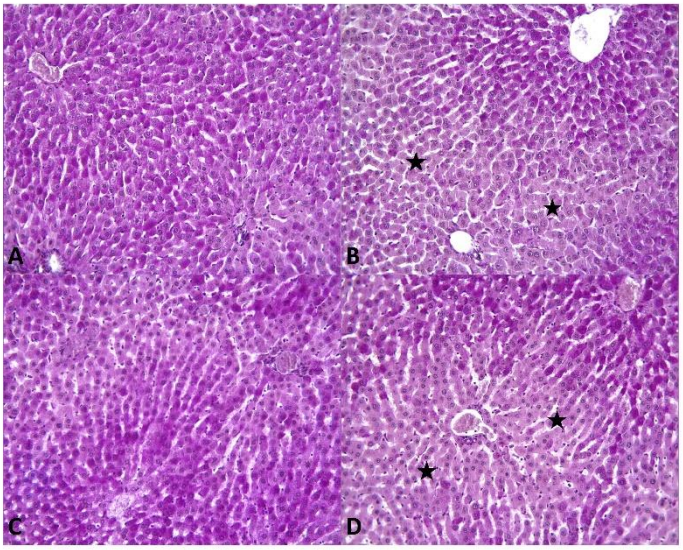

Figure 3. PAS (+) staining in hepatocytes in control group (A) indicates the presence of glycogen. In the CIS group (B), a decrease in PAS (+) staining is observed due to glycogen loss. In the H2S+CIS group (C), PAS (+) staining increased, but glycogen loss was similar to the CIS group in the CIS+H2S group (D).

Discussion

This study aims to reveal both the therapeutic and prophylactic effects of low-dose H2S in the CIS-induced hepatotoxicity and evaluate it biochemically and histopathologically.

CIS, an antineoplastic drug, is widely used in the treatment of breast, cervical, esophageal, bladder, small cell lung, osteosarcoma, squamous cell carcinoma and testicular cancer (Gao et al., 2021; Man et al., 2020). However, the clinical application of CIS is limited due to its serious side effects such as hepatotoxicity (El-Gizawy et al., 2020). Furthermore, because of the harmful effects of ROS by exhausted GSH, antioxidant enzymes like glutathione GPx, SOD, CAT, GSH, and an increase in hepatic MDA, oxidative stress is a major factor in CIS-induced hepatotoxicity (Abd Rashid et al., 2021). In this current study, we focused on the improvement properties of H2S administration in hepatotoxicity caused by CIS.

A single dose of 7.5 mg/kg CIS not only caused an increase in liver function enzymes but also caused histopathological changes in this study. The increase in the level of liver enzymes in the serum indicates that the enzymes pass from the cytosol to the systemic circulation due to the deterioration of the integrity of the liver cell. Similar to the literature, our study observed an increase in liver enzymes (AST, ALT, and LDH) after CIS application, indicating liver damage (Neamatallah et al., 2018; Ijaz et al., 2020; Taghizadeh et al., 2021). In addition, as a result of histopathological evaluations in liver tissue, it was determined that CIS injection caused sinusoidal dilatation, congestion, necrosis, and glycogen loss. All these findings were in agreement with previous studies (El-Gizawy et al., 2020; Wang et al., 2018; Sherif, 2021). The integrity of the sinusoidal structure is extremely important in that it plays an important role in the continuous physiological exchange of metabolites and fluids in the liver. Disruption of this structure reflects the impairment of liver function (Ahmed, 2013). Also, similar to the literature, a decrease in body weight and liver tissue weight was detected in the CIS group compared to the control group (Ko et al., 2014; Maheshwari et al., 2015).

According to previous studies, it has been revealed that the use of some natural compounds and products improves both biochemical and histopathological damage caused by liver toxicity (El-Gizawy et al., 2020; Wang et al., 2018; Sherif, 2021). Our present findings showed that H2S provides significant improvements in histopathological deterioration in the hepatotoxicity model induced by CIS. In addition, H2S has been shown to have hepatoprotective and therapeutic effects. Also, the use of NaHS, an H2S donor, caused a significant decrease in liver enzymes and statistically significantly improved the histopathological damage caused by CIS in the liver. By replenishing the amount of antioxidant enzymes and functioning as an anti-inflammatory agent, a variety of natural products, plant extracts, and oil rich in flavonoids, terpenoids, polyphenols, and phenolic acids were able to reduce oxidative stress. Similarly, following CIS administration, honey and royal jelly therapy was shown to reduce serum transaminases and scavenge free radicals in the liver. These natural items' medicinal qualities show promise as a supplemental treatment to combat the hepatotoxicity caused by CIS (Abd Rashid et al., 2021).

The liver plays a central role in preventing damage caused by ROS and eliminating ROS products due to its detoxification properties and antioxidant capacity (Koroglu et al., 2021). Although there are many mechanisms underlying the damage caused by CIS in the liver, oxidative stress and decreased antioxidant capacity are the most important mechanisms (Al-Malki & Sayed, 2014). ROS, which is produced much more than the antioxidant capacity, shows many effects in the cell. These effects include an increase in cell death, a decrease in proliferation as well as an increase in lipid peroxidation (Gunata & Parlakpinar, 2021). All these changes affect the signaling pathways in the cell and cause hepatocyte damage (El-

Gizawy et al., 2020; Auten & Davis, 2009). It is known that CIS causes toxicity by increasing intracellular reactive oxygen and nitrogen species in the liver (Chirino et al., 2008). Overproduced free radicals damage not only intracellular structures but also highly unsaturated fatty acids in the cell membrane and may increase lipid peroxidation levels. Data from this study are consistent with previous studies supporting oxidative stress and lipid peroxidation (Fathy et al., 2022; Gunata & Parlakpinar, 2021; Chirino et al., 2008; Farooqui et al., 2016; Eisa et al., 2021).

In accordance with the results of the study in the literature, it was shown that CIS caused a significant decrease in the level of antioxidant enzymes such as CAT and non-enzymatic antioxidant GSH in the liver tissue. It caused a significant MDA value increase, indicating lipid peroxidation (Fathy et al., 2022; Taghizadeh et al., 2021; Sioud et al., 2020; Aboraya et al., 2022; Bilgic et al., 2018). MDA, which is accepted as a marker for oxidative stress, is a lipid peroxidation product and an increase in its level is an indicator of liver damage (Ko et al., 2014). In addition, the most important components of antioxidant defense in the intracellular structure include SOD, which catalyzes the conversion of superoxide anion radical to hydrogen peroxide and oxygen, and CAT, which hydrolyzes hydrogen peroxide to water and oxygen (Sherif, 2021). In addition, GSH has an antioxidant effect by interacting directly with ROS through its sulfhydryl group (Ko et al., 2014). In addition, reduced glutathione, known as an antioxidant, is an important endogenous antioxidant that acts by scavenging free radicals directly or through some antioxidant enzymes (Birk et al., 2013). Accordingly, the level of GSH in the CIS-administered group decreased as a result of its consumption in the scavenging of ROS and nitrogen species produced by CIS in this study. In addition, CIS causes a GSH-platinum complex to form upon entry into the cells (Chu, 1994). This causes a decrease in the level of GSH. The reason for the increase in the level of GSH in the liver may be the increase in the activity or expression of glutamylcysteine ligase, the rate-limiting enzyme in GSH synthesis (Dickinson et al., 2003). Activation of all these enzymes following CIS application leads to the formation of high levels of free radicals. Usually, ROS elimination occurs depending on the liver's antioxidant capacity (Koroglu et al., 2021). However, excess oxidant products from CIS administration exceed the antioxidant capacity of the liver. The reason for the increase in the level of GSH in the liver may be the increase in the activity or expression of glutamylcysteine ligase, the rate-limiting enzyme in GSH synthesis (Dickinson et al., 2003). However, there was no significant difference in SOD and GSH-Px values between all groups.

It has been shown that H2S improves the levels of antioxidant enzymes and non-enzyme CAT and GSH in the liver and reduces lipid peroxidation by causing a decrease in MDA value in CIS-induced hepatotoxicity. Previous studies have shown that H2S effectively scavenges ROS such as H2O2, O2•, and •OH and inhibits NADPH oxidase, which is the main source of ROS overproduction (Wang et al., 2019; Bitar et al., 2018). This may also explain the effect of H2S in current research, where it has been shown that H2S provides a significant decrease in hepatic MDA levels, the end product of lipid peroxidation, an increase in CAT, GSH levels, improvement in liver function enzymes (AST, ALT, LDH) and histopathological improvement.

Light microscopy was used to detect morphological changes in the liver. Our histopathological results showed that CIS treatment caused sinusoidal dilatation, congestion and necrosis. In addition, PAS staining was also performed, and it was observed that PAS (+) areas decreased due to glycogen loss in the CIS group. These effects are similar to the results of previous studies in the literature (Fathy et al., 2022; Eisa et al., 2021; Fatima et al., 2021; Coskun et al., 2021; Cagin et al., 2015). All of these findings explain the hepatotoxicity pathophysiology of CIS. On the contrary, it was determined that H2S treatment caused a statistically significant improvement in histopathological damage and PAS staining in both the prophylactic and treatment groups. This finding supports that HS treatment may have potent antioxidant effects in CIS-induced hepatotoxicity in rats. In conclusion, histopathological evidence supports our biochemical findings.

The normalization of these values in the prophylactic and therapeutic groups indicates that H2S has an antioxidant effect and has positive effects on liver damage caused by CIS. However, the protective and therapeutic efficacy of H2S was first reported in this study by histopathological and biochemical analyzes in the CIS-induced hepatotoxicity model.

Conclusion

It has been shown that HS provides significant improvement in liver function enzymes (AST, ALT, and LDH), tissue antioxidant capacity (MDA, CAT, and GSH), and improvement in histopathological damage (sinusoidal dilatation, congestion, necrosis, and glycogen loss). The administration of H2S showed both prophylactic and therapeutic effects in the experimental model of hepatotoxicity induced by CIS. Therefore, the findings of

the study should be supported by further clinical studies. Our experimental study results need to be supported by further clinical studies.

Ethical approval: The study protocol was approved by the Ethics Committee on Animal Research (reference no: 2015/A-84 – 22.10.2015) under the Faculty of Medicine, Inonu University, Malatya, Turkey.

Peer-review: Externally peer-reviewed.

Author Contributions: KEB, OO, RB, AA, and HP are the coordinators of this study and they planned the study protocol design. KEB, MG, OO, RB, and HP made the mandatory requirements for the study. KEB and OO were responsible for drug administration. OO and MG were responsible for data collection. KEB, OO and HP performed the surgical procedures. YT conducted the biochemical analyses. The histopathological evaluations carried out by NV and AY whereas AY and MG performed statistical analysis. MG and HP were responsible for interpretation of the results. MG was responsible for the design of figures and tables. This manuscript was written by MG, OO, and HP. The final manuscript is revised collaboratively by MG and HP.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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Evaluation of Information on Pregnancy and Lactation in Patient Information Leaflets of Antirheumatic Drugs

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ABSTRACT

Objective: The use of antirheumatic drugs during pregnancy and lactation should be arranged so as not to harm the fetus and mother. There are various sources of information, such as pregnancy guidelines and patient information leaflet (PIL), which are used to determine the course of treatment during these periods.
Methods: In this study, different antirheumatic drug PILs were evaluated in terms of the inconsistency of their information on the use of drugs during pregnancy and lactation with the current European League Against Rheumatism (EULAR)' guidelines. Inconsistent PILs were categorized into 2 different groups according to their information on the use of the drug during pregnancy and lactation: I. lack of information and II. Conflict or contrary of information.
Results: It was found that 72.1% of the 179 PILs had inconsistent pregnancy information (lack of information n=22, conflict or contrary of information n=107) and 75.4% had inconsistent lactation information (lack of information n=22, conflict or contrary of information n=113) compared with EULAR. According to the EULAR, 79.3% (n=142) of the drugs in this study were suitable for use in pregnancy and 80.4% (n=144) were suitable for use during lactation.
Conclusion: The findings of this study draw attention to the need for national and international regulation to harmonize the information in PILs with the current literature. This study will be useful for patients and healthcare professionals to ensure that PILs are compatible with the current literature.

Key words: Antirheumatic drug, Lactation, Patient information leaflet, Pregnancy.

Introduction

Rheumatic diseases are more likely to affect women of childbearing age (Giles et al., 2019). Pregnant women with rheumatic diseases are at higher risk of maternal and neonatal complications than the general pregnant population (He & Wei, 2020; Sim et al., 2023). These patients may become pregnant while taking drug or may need to take drug during pregnancy and/or lactation. For a healthy pregnancy, the drug status of these patients should be adjusted before pregnancy, such as discontinuing known or potentially teratogenic drugs and/or switching to appropriate drugs. On the other hand, the control of disease activity in rheumatic diseases should be performed carefully during pregnancy and lactation. Drugs used in the treatment of rheumatological diseases known to be compatible with pregnancy and lactation should be given to the mother at the lowest possible dose, with the aim of avoiding harm to the fetus and baby (Brooks & Needs, 1990).

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Guidelines and recommendations about antirheumatic drug use in pregnancy and lactation are periodically published which became scientific sources that enable healthcare professionals and patients to access up-to-date information. The European League Against Rheumatism (EULAR) has compiled available data on antirheumatic drugs from the literature and various databases and has developed an expert consensus on the compatibility of these drugs during pregnancy and lactation, and considerations for their use during these periods (Götestam Skorpen et al., 2016). There are differences between these sources of information on drug use in pregnancy in terms of the usability of drugs (Birru Talabi & Clowse, 2020; Flint et al., 2016).

In a study of patients with rheumatic diseases, 84% of patients reported receiving information about the drug from patient information leaflets (PILs), 80% from rheumatologists and 50% from general practitioners (Ørnbjerg et al., 2008). However, the information given by guidelines and authorities may sometimes contradict by the information supplied by PILs. Lack of up-to-date information concerning the use of antirheumatic drugs during pregnancy and lactation may lead to unable to manage the treatment that leads to unnecessary discontinuation or switching of drugs (Desai et al., 2016; Flint et al., 2016).

As in the whole world, there is a PIL in every pharmaceutical preparation in Turkiye including the antirheumatic drugs, for giving essential information about the drugs to the patients. Those PILs include pharmaceutical contents, therapeutic indications, contraindications, precautions, how it is used, possible side effects, storage conditions, contents of the package and some other related information in Turkiye (Turkish Medicines and Medical Devices Agency, 2008).

In this study, different antirheumatic drug PILs were evaluated for inconsistencies in their information on the use of the drugs during pregnancy and lactation with the current EULAR recommendations.

Methods

This study compares the EULAR recommendations on the use of antirheumatic drugs in the period of pregnancy and lactation, published by the EULAR Task Force in 2016, with the information provided in the PILs of antirheumatic drugs (originator and generics, if available) approved in Turkey. The PILs were obtained from the website of the Turkish Medicines and Medical Devices Agency (TITCK), the Turkish national official regulatory authority for medicines and medical devices. Only the PILs of the antirheumatic drugs approved in Turkiye (both originator and generics) have been included in this study. Additionally, the preparations which have only those antirheumatic agents were included and any combination of those agents were excluded in this study. Likewise, topically administered preparations were also excluded. Orally or parenterally administered antirheumatic preparations' PILs were evaluated.

In this study, the PILs were classified into 3 different groups according to the information they provided on the use of the drug in the period of pregnancy and lactation, as follows: I. lack of information, II. conflict or contrary information and III. consistent with EULAR recommendations. Thus, the PILs included in the first and second group are accepted as having inconsistencies and the details of inconsistencies between the EULAR recommendations and PILs were:

Although EULAR recommendations indicate that the use of drug is compatible during pregnancy and lactation but if the PILs:

a) if there is a warning such as "Before using this drug, tell your doctor if you are pregnant, trying to become pregnant, or are lactating", "Consult your doctor or pharmacist before using the drug" and "If you notice that you are pregnant during your treatment, consult your doctor or pharmacist immediately" or

b) did not state any definite recommendation about the agent's use during pregnancy and lactation even it is expressed as "human and/or animal data identifies transplacental passage, excretion into breast milk or cause adverse outcome" (accepted as "type I inconsistency").

Although EULAR recommendations indicate the use of drug is compatible during pregnancy and lactation, but the PIL restricts, contraindicates or not recommends the use during pregnancy and lactation (accepted as "type II inconsistency").

We also evaluated the PILs of antirheumatic pharmaceutical preparations that were approved by TITCK before or after the publishing date of EULAR recommendations which was 17 February 2016. PILs and EULAR recommendations were reviewed by both authors (N.C.D., A.K.). The study examined interrater consensus by calculating Cohen's kappa coefficient. The third author

(N.I.) was the decision-maker in cases of disagreement between the other authors. Descriptive statistics and kappa coefficient calculations were performed using Microsoft Excel 2024 software for statistical analysis.

Results

Inter-rater agreement

Cohen's kappa coefficient was calculated as 0.824 for pregnancy and 0.874 for lactation, indicating high inter-rater agreement. There were 28 PILs that could not be agreed between the two authors and these PILs were evaluated by the third author.

Agents

One hundred seventy nine PILs of antirheumatic preparations having 31 different pharmacologically active substances were analyzed: 4 nonsteroidal anti-inflammatory agents (aspirin, diclofenac, ibuprofen, naproxen), 1 selective COX-2 inhibitor (celecoxib), 3 glucocorticoids (methylprednisolone, prednisolone, prednisone), colchicine, 5 DMARDs (chloroquine, hydroxychloroquine, leflunomide, methotrexate, sulfasalazine), 5 immunosuppressants (azathioprine, cyclophosphamide, cyclosporine, mycophenolate, tacrolimus,), intravenous immunoglobulin (IVIG), 10 biopharmaceutical agents (abatacept, adalimumab, belimumab, certolizumab, etanercept, infliximab, golimumab, rituximab, tocilizumab, ustekinumab), and tofacitinib.

Pregnancy information

Out of the 179 PILs, 72.1% presented information inconsistencies about the drugs' use during pregnancy. On the other hand, 27.9% of the PILs (n=50) have consistency. The PILs that have inconsistencies about the drugs' use during pregnancy period regarding EULAR recommendations are of acetyl salicylic acid, diclofenac, ibuprofen, naproxen, methylprednisolone, colchicine, prednisolone, prednisone, chloroquine, hydroxychloroquine, sulfasalazine, azathioprine, cyclosporine, tacrolimus, intravenous immunoglobulin, adalimumab, etanercept, infliximab, rituximab and golimumab (20 out of 31 different agents).

The reasons for inconsistencies are the lack of information in 12.3% of the PILs (n=22) and 59.8% (n=107) where the information is in conflict with or contrary to the EULAR recommendations. Before EULAR, 71 PILs were issued. The reasons for inconsistencies are the lack of information in 16.9% of the PILs (n=12) and 57.8% (n=41) where the information is in conflict with or contrary to the EULAR recommendations. The amount of consistency is 25.3% of the PILs (n=18). After EULAR, 108 PILs were issued. The reasons for inconsistencies are the lack of information in 9.3% of the PILs (n=10) and 61.1% (n=66) where the information is in conflict with or contrary to the EULAR recommendations. The reasons for consistencies are 29.6% of the PILs (n=32). After the EULAR recommendations released in 2016, 108 of the 179 drugs were revised in their PILs or newly registered to the market and 4.3% become compliant, was shown in Table 1.

Table 1. Analysis of consistencies and inconsistencies between the EULAR recommendations and PILs for drug use during pregnancy and lactation

	Inconsistent				**Consistent**	
	Lack of information		**Conflict or contrary information**			
	Pregnancy n (%)	**Lactation** n (%)	**Pregnancy** n (%)	**Lactation** n (%)	**Pregnancy** n (%)	**Lactation** n (%)
All (n=179)	22 (12,3)	22 (12,3)	107 (59,8)	113 (63,1)	50 (27,9)	44 (24,6)
Before EULAR (n=71)	12 (16,9)	12 (16,9)	41 (57,8)	44 (62)	18 (25,3)	15 (21,1)
After EULAR (n=108)	10 (9,3)	10 (9,3)	66 (61,1)	69 (63,9)	32 (29,6)	29 (26,8)

EULAR: European League Against Rheumatism, PIL: Patient information leaflet

Some of the PILs of generic preparations of naproxen, prednisolone and tacrolimus had different information than the generics about pregnancy period, which was shown in Table 2.

Table 2. Classification of PILs compared to EULAR recommendations in 3 different groups according to their information during pregnancy

Consistent	Naproxen*, methotrexate, mycophenolate, cyclophosphamide, leflunomide, celecoxib, certolizumab, golimumab, abatacept, tocilizumab, belimumab, ustekinumab, tofacitinib
Lack of information	Ibuprofen, methylprednisolone, prednisolone*, tacrolimus*, intravenous immunoglobulin, rituximab
Conflict or contrary information to EULAR	Aspirin, diclofenac, ibuprofen, naproxen*, prednisolone*, prednisone chloroquine, hydroxychloroquine, sulfasalazine, azathioprine, cyclosporine, tacrolimus*, colchicine, adalimumab, etanercept, infliximab

EULAR: European League Against Rheumatism, PIL: Patient information leaflet

*Some of the PILs of generic preparations of naproxen, prednisolone and tacrolimus had different information than the generics (i.e., the information of two of naproxen preparations' PILs were consistent with EULAR recommendations while remaining 25 PILs' information were conflicting regarding the drugs' use during pregnancy).

Drugs suitable for use in pregnancy

According to EULAR recommendations, 79.3% (n=142) of the drugs included in this study are suitable to use during pregnancy. There is clear information that only one-tenth of the PILs of these drugs (n=15), that are suitable for use during pregnancy are used during pregnancy.

Lactation information

When we focused on the information given by the PILs about the agents' use during lactation, 135 of 179 (75.4%) also have inconsistencies with the EULAR recommendations. The PILs that have inconsistencies about the drugs' use lactation period regarding EULAR recommendations are of acetyl salicylic acid, diclofenac, ibuprofen, naproxen, celecoxib, methylprednisolone, colchicine, prednisolone, prednisone, chloroquine, hydroxychloroquine, azathioprine, sulfasalazine, tacrolimus, cyclosporine, intravenous immunoglobulin, adalimumab, etanercept, infliximab, golimumab and tofasitinib (21 out of 31 different agents).

The reasons for inconsistencies are the lack of information in 12.3% of the cases (n=22) and 63.1% of cases (n=113) where the information is in conflict with or contrary to the EULAR recommendations. Before EULAR, 71 PILs were issued. The reasons for inconsistencies are the lack of information in 16.9% of the PILs (n=12) and 62% (n=44) where the information is in conflict with or contrary to the EULAR recommendations. The amount of consistency is 21.1% of the PILs (n=15). After EULAR, 108 PILs were issued. The reasons for inconsistencies are the lack of information in 9.3% of the PILs (n=10) and 63.9% (n=69) where the information is in conflict with or contrary to the EULAR recommendations. The amount of consistency is 26.8% of the PILs (n=29). After the EULAR recommendations released in 2016, 108 of the 179 drugs were revised in their PILs and 5.7% become compliant, was shown in Table 1.

Some of the PILs of generic preparations of naproxen, ibuprofen, prednisolone, and intravenous immunoglobulin had different information than the generics about lactation period was shown in Table 3.

Table 3. Classification of PILs compared to EULAR recommendations in 3 different groups according to their information during lactation

Consistent	Prednisone, methotrexate, mycophenolate, cyclophosphamide, leflunomide, intravenous immunoglobulin*, abatacept, belimumab, certolizumab, golimumab, rituximab, tocilizumab, tofacitinib
Lack of information	Ibuprofen*, naproxen*, methylprednisolone, prednisolone*, colchicine, intravenous immunoglobulin*, ustekinumab
Conflict or contrary information to EULAR	Aspirin, diclofenac, ibuprofen*, naproxen*, prednisolone*, celecoxib, chloroquine, hydroxychloroquine, sulfasalazine, azathioprine, cyclosporine, tacrolimus, adalimumab, etanercept, infliximab

EULAR: European League Against Rheumatism, PIL: Patient information leaflet

*Some of the PILs of generic preparations of naproxen, ibuprofen, prednisolone, and intravenous immunoglobulin were having different information than the generics (i.e., the information of one of intravenous immunoglobulin preparation's PIL was consistent with EULAR recommendations while remaining 10 PILs' information were having lack of information regarding the drugs' use during lactation).

Drugs suitable for use in lactation

According to EULAR recommendations, 80.4% (n=144) of the drugs in this study are suitable for use in lactation. There is clear information that these drugs, which are suitable for use in lactation, were stated to be suitable for use during lactation in 6.9% (n=10) of PILs**.**

PIL active ingredients compatible with EULAR both during pregnancy and lactation

PILs (Originator and all generics, if any) that contain information compatible with EULAR in both pregnancy and lactation consist of methotrexate, mycophenolate, cyclophosphamide, abatacept, belimumab, leflunomide, tocilizumab, ustekinumab which should not be used during pregnancy and lactation and certolizumab that can be used during these periods (n=40, 22.3%).

There are 15 PILs in this study of methotrexate, mycophenolate mofetil and cyclophosphamide, which are known to be teratogenic and should be discontinued before pregnancy according to EULAR. The pregnancy and lactation information of these PILs is in line with EULAR.

Discussion

Little research has investigated the discrepancy between the PILs of antirheumatic drugs and the pregnancy and lactation information of scientific sources.

In the present study, we used the EULAR recommendations, which is a current source, to determine the differences in pregnancy and lactation information in PILs of antirheumatic drugs in Turkiye. Current study results revealed that only 27.9% of the PILs' pregnancy information contains current drug guidance information whereas the remaining 72.1% is inconsistent to EULAR recommendations. In another study performed in Argentina the inconsistency ratio was half of PILs as to pregnancy (Sabando et al., 2018). Although patients, guidelines and the drugs are the same, this shows that the information in the PILs' of antirheumatic preparations do not contain standard information against the countries. In a study comparing PIL and established clinical resources of 245 drugs, it was stated that 75.9% of pregnancy information and 87.3% of lactation information were inconsistent (Brown et al., 2016). In addition, the availability of pregnancy and lactation information in PIL varies from country to country (Arsalan et al., 2015; Hailu et al., 2022; Khamas et al., 2019).

The difference of this study is the evaluation of PILs according to the publication date of the scientific source. 39.7% (n=71) of the drugs in current study were approved for PIL before EULAR recommendations. When approved PILs were evaluated after the ER was published, PILs were found to be inconsistent at a rate of 71.4% during pregnancy and 73.2% during lactation. In current study, it was determined that after the publication of the EULAR recommendations, PIL compliance increased by 4.3% during pregnancy and 5.7% during lactation. This increase is not sufficient and PILs, which are one of the drug information sources of patients, should be updated by companies on the basis of scientific resources. The current study does not explain whether this increase is due to EULAR recommendations. Regulations in accordance with the guidelines are important in terms of patient compliance and trust in the health authority.

Most of the drugs in this study were compatible with pregnancy and lactation according to the EULAR recommendations (n=142 and n=144, respectively). It was determined that 89.4% of the drugs compatible with pregnancy did not have information compatible with pregnancy. This rate is 93.1% for lactation. In one study, it was stated that inconsistent information about pregnancy and lactation confuses patients (Bjerrum & Foged, 2003). The confusion caused by this inconsistency may lead to unnecessary drug discontinuation and cessation of lactation in rheumatology patients (Ince-Askan et al., 2019; Kemper et al., 2022; Rebić et al., 2020). There are studies showing that discontinuation of drugs in pregnant women with rheumatic disease causes exacerbation of the disease (Gerardi et al., 2022; van den Brandt et al., 2017).

In a study, it was stated that PILs containing the same active ingredient contain inconsistent information with each other and the guidelines (Crunkhorn et al., 2017). Also in this study, the fact that different preparations of the same active substance contain different information may cause patients to distrust health authority (naproxen, ibuprofen, prednisolone, tacrolimus, and intravenous immunoglobulin). A study showed that inconsistency and different information from different PILs involving the same active substance can lead to confusion among patients (Bjerrum & Foged, 2003). PILs containing different information on these drugs may reduce confidence in the health authority that approves them.

Precautions especially for pregnancy and lactation should be included in PILs, which are the most important document of information about a medicine that patients have access (Arsalan et al., 2015). 12.3% of the PILs in this study contain general information that does not mention the risk in pregnancy and lactation. According to the European Medicines Agency 2009 publication on consumer expectations, PILs should clearly describe the drug's benefits on the one hand and the drug's risks on the other, for pregnant women (European Medicines Agency, 2009).

The results of this study indicate that there is a national and international need for the information in the PIL to be in harmony with the current literature. Current study will be beneficial for patients and healthcare professionals to ensure that PILs are compatible with the current literature. Current study protocol can be developed and used in new studies where the pregnancy and lactation information of PILs related to different drug groups are compared with scientific sources.

This study has potential limitations. The first is to compare with a single scientific source (EULAR). The findings in the study were created because of personal evaluations. Although this seems like a limitation, we believe that the presence of a rheumatologist and two pharmacologists in the evaluation team who have studied drug use during pregnancy and lactation contributes to the analysis of the differences in the contents of PILs.

Conclusions and Recommendations

The information in the PILs of the drugs such as hydroxychloroquine, chloroquine, sulfasalazine, azathioprine, cyclosporine, tacrolimus, colchicine about the use of the drugs in some or all periods of pregnancy are either restricted or not recommended although they were expressed to be safe and compatible by the EULAR recommendations. Therefore, this situation has the potential to create a negative opinion against rheumatology specialists who follow the literature regularly, both in patients and non-specialist physicians.

The data we obtained from this study showed that for two-thirds of the pregnancy and lactation information (61.1% of pregnancy and 63.9% of lactation) in PILs of antirheumatic drugs had conflicting information with that in the EULAR recommendations. These differences can lead to ambiguity and potential risks, especially for patients and healthcare professionals. The findings obtained in the study indicate that there is a need for more guidance and standardization on the content of PIL information. Information must be compatible between regulatory agencies and scientific sources. According to the literature, the problem of heterogeneous drug information is not limited to Turkey and that current study results should be evaluated internationally.

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A Randomised Controlled Trial to Evaluate Genotyping and Therapeutic Drug Monitoring *vs* Only Therapeutic Drug Monitoring as a Strategy for Risk Minimisation in Epileptic Patients on Carbamazepine Therapy

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ABSTRACT

Objective: Carbamazepine (CBZ) is a widely prescribed antiepileptic drug for the treatment of focal seizures. CBZ is metabolised primarily by cytochrome enzymes, particularly CYP3A5. It is difficult to predict clinically whether a patient is likely to suffer from CBZ toxicity. Hence, we aimed to evaluate the use of genotyping and therapeutic drug monitoring (TDM) vs. only TDM in epileptic patients on CBZ as a strategy for risk minimisation.

Methods: This double-blind, randomised controlled trial included 60 patients with epilepsy who were receiving carbamazepine. They were randomly assigned to two equal groups, with one group's carbamazepine dosing guided by genotyping and the other group's doses based solely on clinical judgement.

Results: A total of 60 patients were enrolled in the study and allocated into two groups, group A (both genotyping and TDM) and Group B (only TDM), each arm comprising 30 patients. Among the CYP3A5 metaboliser group, the frequency of expressors and non-expressors was 57% and 43%, respectively. During follow-up visits, at one month, three cases of adverse drug reactions (ADRs) were reported. The number of ADRs decreased to two at the three-month follow-up and declined to a single case at the 12-month assessment. It was found that there is no statistically significant association between CYP3A5 metaboliser and ADR occurrence.

Conclusion: Adding genotyping to TDM did not significantly reduce the risk of carbamazepine toxicity. However, genotyping may still be useful for patients who exhibit symptoms of toxicity.

Keywords: Carbamazepine, Drug Monitoring, Drug-Related Side Effects and Adverse Reactions, Genetic polymorphism, Risk Evaluation and Mitigation

Introduction

Carbamazepine is generally used in patients with generalised tonic-clonic seizures and focal seizures. It is also used for the treatment of bipolar disorders. It shows several "idiosyncratic" adverse effects requiring termination of the therapy (Potter & Ketter, 1993). For the treatment of seizures and bipolar disorders, serum carbamazepine concentrations of 4-12 µg/ml are considered within the accepted therapeutic range (van Tyle & Winter, 2004).

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Pharmacogenetics is the study of the relationship between variations in a single gene and the action of drugs, and these genetic distinctions aid in clarifying the reason for either treatment failure or toxic effects related to several pharmaceutical compounds. It is important to know about these distinctions for the prognosis of the incidence of toxicity amongst patients receiving any drug (Cavalleri et al., 2011). Genetic polymorphisms that occur because of a single-nucleotide exchange in the DNA sequence are more common. Genetic polymorphisms show a significant role in the variability in pharmacokinetics and pharmacodynamics of anti-epileptic drugs (AEDs) and can affect their efficacy, tolerability, safety, and duration of action (Roden, 2006; Seven et al., 2014; Lakhan et al., 2011; Orozco-Suarez, 2014; Franco & Perucca, 2015).

The cytochrome P450 enzymes are involved in the metabolism and elimination of numerous extensively used drugs and are very much predisposed to genetic polymorphism. Carbamazepine elimination is linked to genetic polymorphisms of drug-metabolising enzymes and transporters (Puranik et al., 2013; Yeap et al., 2014). Ninety-nine percent of carbamazepine is metabolised by the liver, and CYP3A4 and CYP3A5 are the most prominent enzymes. Amongst the patients getting the same dose of carbamazepine, it shows a noticeable interindividual distinction in the plasma drug concentrations, which may lead to therapeutic failure or toxicity. It is realised that there are noteworthy inter-individual variances in the expression of CYP3A5, thus showing distinctions in the pharmacokinetics of carbamazepine (Perucca, 2006; Thorn et al., 2011).

Carbamazepine is cleared from the blood at a higher rate of about 8% in patients with CYP3A5*3/*3 alleles as compared to those with CYP3A5*1/*1 or CYP3A5*1/*3 alleles, as reported by Seo et al. (Seo et al., 2006). However, Park et al. (Park et al., 2009) reported that there were higher levels in patients with CYP3A5*3/*3, which were 31% more than those in patients with CYP3A5*1/*1 or CYP3A5*1/*3. Detection of plasma levels is a routine practice that serves as a guide to regulate the titration of doses. It aids in lessening the risk of under- or overdosing due to drug/ food interaction or genetic polymorphism of enzymes and transporters involved in the metabolism of carbamazepine (Raj Panday et al., 2017). A study conducted by Al-Gahtany et. al. (2014) recommended that the CYP3A5 genetic polymorphisms result in toxicity in epileptic patients by playing an important role in the steady-state concentrations of carbamazepine.

Carbamazepine serum disposition is changed by the genetic polymorphisms of metabolic enzymes, necessitating therapeutic dose monitoring. It is almost fully metabolised in the liver, with only approximately 5% of the drug excreted unchanged. Among the diverse types of alleles of CYP3A5, the frequently occurring type, which leads to loss of its function, is the CYP3A5*3 allele. Thus, only people with at least one CYP3A5*1 allele can express large amounts of CYP3A5 (expressors), while individuals homozygous for the mutant allele CYP3A5*3/*3 are considered non-expressors (Milovanovic et al., 2015; Barry & Levine, 2010).

A risk management plan is defined as “a set of pharmacovigilance events and interventions planned to detect, describe, avert or diminish risks relating to medicinal products, including the assessment of the effectiveness of those interventions” (Touw et al., 2005). Carbamazepine produces dose-related neurotoxicity such as sedation, dizziness, vertigo, diplopia, and ataxia. Vomiting, diarrhoea, and worsening of seizures are also seen with higher doses. It can also cause specific rare side effects, including severe cutaneous adverse reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis (Thorn et al., 2011). In addition to adverse events, the absence of efficacy can also be a problem, with as many as 30% of patients with epilepsy facing drug resistance (Sisodiya & Goldstein, 2007).

It is hard to envisage clinically whether a patient is likely to suffer from carbamazepine toxicity. One of the ways to avoid the adverse effects of drugs is through therapeutic drug monitoring (TDM), as it aids in distinguishing between drug toxicity and uninhibited disease for some drugs. TDM aids in speeding up the establishment of a drug regimen for an individual patient. When TDM is performed, the therapeutic ranges that have been established for the drugs in the class should be used only as guides. Genotyping, on the other hand, can aid in personalising carbamazepine therapy by detecting mutations in the enzymes responsible for its metabolism, thus envisaging the dose range for a given drug to circumvent toxicity in the patient. Hence, this study was conducted to compare the addition of genotyping to TDM of carbamazepine as a new tool and assess the plasma levels of carbamazepine as well as the occurrence of ADRs in epileptic patients on carbamazepine therapy as a part of risk minimisation.

Methods

Trial design

The study was a prospective, parallel, double-blind, randomised controlled trial conducted in two groups of epileptic patients on carbamazepine therapy recruited from either the Neurology or Therapeutic Drug Monitoring (TDM) outpatient department (OPD) of a tertiary care teaching hospital in India. It was conducted as per the Indian Council of Medical Research (ICMR) guidelines 2017, and it was approved by the Institutional Ethics Committee [EC/OA-41/2019]. Clinical Trial Registry of India (CTRI) registration was done with the registration number CTRI/2019/09/021311. Written informed consent was obtained from all participants in this study after ethics committee approval. A randomised controlled trial was chosen to compare the efficacy of genotyping-based dosing with standard clinical judgement.

Participants

All patients aged 5 to 85 years, not exposed to carbamazepine therapy in the last year (regardless of monotherapy or polytherapy), were included in the study. Patients with genotyping results (for CYP3A5 polymorphism) known due to prior testing or reports being available in medical records, patients with a history of drug/alcohol abuse, and those with evidence of gastrointestinal tract, renal, endocrine, cardiovascular diseases, etc., and patients with status epilepticus were excluded.

Interventions

After obtaining written informed consent/assent, the patients were randomised into two study arms, with one arm (group A) receiving the therapeutic dose of carbamazepine based on their CYP3A5 genotyping. CYP3A5 expressors (*1/*1 and *1/*3) were given a starting dose of carbamazepine of up to 400 mg twice daily, while CYP3A5 non-expressors (*3/*3) were given a starting dose, as per the neurologist's opinion, up to a maximum dose of 200 mg twice daily. In another study arm (group B), genotyping was not performed initially, and dose administration was based on the clinician's judgement. In both groups, monitoring of carbamazepine levels was performed through TDM. For the patients in group B, genotyping was done after the last follow-up visit to compare the incidence of adverse effects in each group based on their genotypes.

TDM of carbamazepine in both the study arms was done through the estimation of trough levels of carbamazepine concentrations. Trough levels were ensured by educating the patient to visit the OPD before taking the morning dose. Sample collection was done after the completion of 12 hours since the last dosing. Four millilitres (ml) of venous blood samples were collected under aseptic precautions. Samples were centrifuged for the separation of plasma, which was used for estimating the trough levels of plasma carbamazepine using fast elution high-performance liquid chromatography (HPLC) by Chromaster, Japan. Running three-level control sera provided along with the kits ensured maintenance of quality control. These results of carbamazepine TDM level concentrations performed prospectively for one year (1-, 3-, 6- and 12-month follow-up visits) in epileptic patients of either study arm were recorded in case record form (CRF). For DNA extraction and genotyping, the remaining cellular component was stored at −80°C.

Genotyping studies were carried out on these DNA samples after standardising the Polymerase Chain Reaction (PCR) for various parameters such as the DNA and the primer concentrations, dNTPs, $MgCl_2$, and annealing temperature. After optimising the reaction conditions, the DNA samples of the subjects were amplified using primers specific to CYP3A5*3 polymorphisms. The amplified product obtained was then subjected to restriction digestion using a specific restriction enzyme. The product thus obtained was subjected to gel electrophoresis to identify the polymorphism (Adithan et al., 2003; Sullivan-Klose et al., 1996). After obtaining genotyping results in both groups, they were compared with the plasma carbamazepine levels of the participants.

Outcomes

Primary outcome: Comparison of plasma levels of carbamazepine with the CYP3A5 genotype

Secondary outcomes:

- Number of ADRs reported by the study participants
- Comparison of ADRs reported by the participants with their genotype

Sample size

During the planning of the study, there were no similar studies conducted previously. Hence, a convenient sample size of 30 per group was considered.

Randomisation

The randomisation plan was generated from http://www.randomization.com. According to this plan, the patients were divided into two blocks of randomisation with an allocation ratio of 1:1. Allocation concealment was done using opaque, sealed envelopes.

Blinding

Both the patients and the observer (junior clinician) were blinded to the intervention received. The observer assessed only the primary outcome measure of estimating plasma levels of carbamazepine. Another observer (senior clinician) was unblinded to the intervention, noted the ADRs in case they occurred in any of the patients and initiated and appropriately titrated the carbamazepine doses.

Statistical methods

The baseline demographic data were summarised using descriptive statistics. Plasma levels of carbamazepine and ADRs were recorded as categorical data and were summarised as frequencies and percentages. The difference between the two study arms regarding the occurrence of ADRs was analysed using the Chi-Squared test. All analyses were done at 5% significance.

Results

The study period was planned to be completed over two years, but was extended due to the COVID-19 pandemic. 60 patients were enrolled in the study, and all of them completed it as shown in Figure 1.

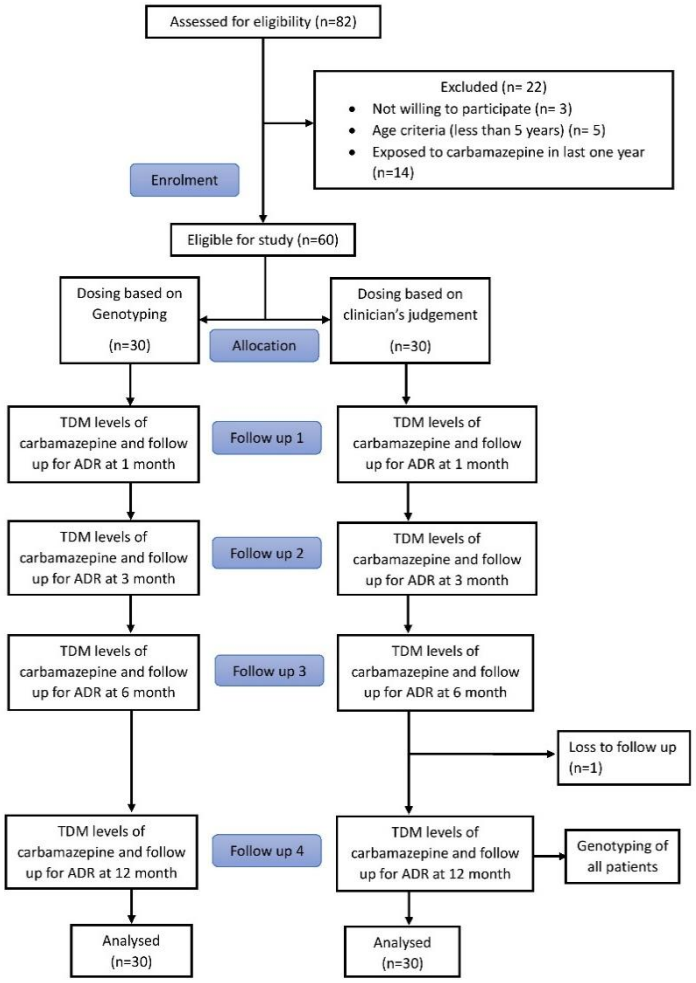

Figure 1. CONSORT flow chart

The baseline characteristics are given in Table 1.

Table 1: Baseline characteristics of study participants

Characteristics		Group A	Group B	*P*
Age (years)		23.21 ± 10.44	24.45 ± 11.44	.67$
Sex	**Male**	12	19	.07#
	Female	18	11	
Weight (kg)		50.66 ± 15.88	55.04 ± 18.10	.33$
Baseline plasma carbamazepine levels (µg/ml)		0.604 ± 0.566	0.433 ± 0.375	.18$

*P*s calculated using unpaired t-test$ and chi-squared test#

Among the CYP3A5 metaboliser group, the frequency of expressors and non-expressors was 34 (56.7%) and 26 (43.3%), respectively. The difference in the mean age of

patients on carbamazepine therapy among CYP3A5 expressors and non-expressors is statistically not significant. To assess the association of CYP3A5 metaboliser status with gender, a chi-square test was used, which indicated that there was no statistically significant association, as depicted in Table 2. Uncontrolled seizures, suspected non-compliance and features of toxicity were the main indications for carrying out TDM in these patients.

Table 2: Difference in gender among CYP3A5 expressors and non-expressors

CYP3A5 metaboliser			
Gender	**Expressers n (%)**	**Non-expressers n (%)**	***P***
Female	15 (51.7)	14 (48.3)	.46
Male	19 (61.3)	12 (38.7)	

P calculated using chi-square test

Table 3 shows the plasma carbamazepine levels over the entire study period.

Table 3: Plasma carbamazepine levels over the entire study period

Follow-up visit (months)	**Group A**	**Group B**	***P***
1	4.602 ± 2.326	4.637 ± 2.274	.95
3	5.604 ± 2.441	5.02 ± 1.468	.28
6	5.13 ± 1.619	5.148 ± 1.125	.96
12	5.432 ± 1.524	5.019 ± 0.898	.22

P calculated using an unpaired t-test

Table 4 shows the association of CYP3A5 metaboliser with the plasma levels of carbamazepine at one, three, six, and 12-month follow-up periods. The results indicate that CYP3A5 metaboliser status was not significantly associated with the plasma levels of carbamazepine during the follow-up periods.

Table 4: TDM levels in CYP3A5 expressors and non-expressors

CYP3A5 metaboliser			
	Expressors n (%)	**Non-expressors n (%)**	***p***
Plasma level of carbamazepine (1-month follow-up) [below/within/above laboratory reference range]			
Below	15 (53.6)	13 (46.4)	.65
Within	19 (59.4)	13 (40.6)	
Plasma level of carbamazepine (3 months follow-up) [below/within/above laboratory reference range]			
Below	8 (66.7)	4 (33.3)	.38
Within	25 (53.2)	22 (46.8)	
Above	1 (100)	0	
Plasma level of carbamazepine (6-month follow-up) [below/within/above laboratory reference range]			
Below	5 (45.5)	6 (54.5)	.51
Within	29 (59.2)	20 (40.8)	
Plasma level of carbamazepine (12-month follow-up) [below/within/above laboratory reference range]			
Below	2 (33.3)	4 (66.7)	.39
Within	32 (59.3)	22 (40.7)	

TDM: Therapeutic Drug Monitoring
*P*s calculated using the chi-square test

During the follow-up visits, at the one-month follow-up period, three cases of ADRs were reported. The ADR count decreased to two cases during the three-month follow-up and decreased to only one case of ADR at the 12-month assessment. These results are shown in Table 5.

Table 5: Number of ADR occurrences in each group

Follow-up visit (months)	**Group A**	**Group B**	***P***
1	2	1	.39
3	1	1	
6	0	0	
12	0	1	

ADR: Adverse Drug Reaction
P calculated using Fischer's exact test

Assessment of the association of CYP3A5 metaboliser status of carbamazepine and ADR indicated that there was no statistically significant association, as depicted in Table 6 below.

Table 6: Occurrence of ADRs in CYP3A5 expressor and non-expressor groups

CYP3A5 metaboliser			
	Expressers n (%)	**Non-expressers n (%)**	***p***
Occurrence of ADR (1-month follow-up)			
No	31 (54.4)	26 (45.6)	.25
Yes	3 (100)	0 (0)	
Occurrence of ADR (3-month follow-up)			
No	32 (55.2)	26 (44.8)	.50
Yes	2 (100)	0 (0)	
Occurrence of ADR (12-month follow-up)			
No	34 (57.6)	25 (42.4)	.43
Yes	0 (0)	1 (100)	

ADR: Adverse Drug Reaction

*P*s calculated using the chi-square test

Discussion

This is the first study conducted on Indian epileptic patients on carbamazepine therapy to find out the utility of TDM and genotyping in risk minimisation. This study will contribute to the literature by providing an improved understanding of CYP3A5 polymorphisms in epilepsy patients on treatment with carbamazepine. Earlier research, including studies from Asian countries such as Japan (Seo et al., 2006), Korea (Park et al., 2009), China (Meng et al., 2011; Lu et al., 2018), and Thailand (Panomvana et al., 2013), studied the association between the CYP3A5 genotypes and the disposition of carbamazepine (Thorn et al., 2011). Whereas this association remains indecisive, no such randomised controlled trial has been conducted in Indian epileptic populations.

In our study, the mean age of patients was 24 years, with a male preponderance. The findings of our study were consistent with the mean age of patients in a study done by Ganesapandian et. al. (2019) in the South Indian population. In our study, we found that among male patients on carbamazepine therapy, 61% were expressors and 39% were non-expressors of CYP3A5, while in the study conducted by Ganesapandian et. al. (2019), they found 45% expressers and 55% non-expressors of CYP3A5 among male patients on carbamazepine therapy. In the case of female patients, we found 52% were expressors and 48% were non-expressors of CYP3A5, while in the same study conducted by Ganesapandian et. al. (2019), they found 55% expressers and 45% non-expressors among female patients. A similar pattern of CYP3A5 expression among both genders in both studies was observed.

In our study, the mean baseline carbamazepine plasma levels at the time of patient enrollment were 0.52 µg/ml, well below the laboratory reference range of 4-12 µg/ml, confirming that the patients had received no treatment. During a monthly follow-up period of patients, we found that the mean plasma carbamazepine levels were within the laboratory reference range in 53% (both arms of patients) after starting carbamazepine therapy. Though the mean plasma levels appeared to be within the laboratory reference range at one-month follow-up, only 59% of expressors and 41% of non-expressors had plasma levels within the laboratory reference range. This may be because carbamazepine, being an effective enzyme inducer, causes autoinduction by stimulating CYP3A4, and it is usually completed within 3–5 weeks (Pynnönen et al., 1980).

In our study during the follow-up period, only one patient on carbamazepine (out of 60 patients enrolled) had plasma levels above the laboratory reference range at three three-month follow-up visits, and he was a CYP3A5 expressor. Nevertheless, it is well known that increased plasma concentration of carbamazepine is detected in non-expressers. The explanation for this is that the CYP3A5*3 allele has a guanine (G) nucleotide instead of an adenosine (A), creating a cryptic splice site in intron 3 and changing the mRNA splicing. This causes early termination of protein synthesis, leading to the production of nonfunctional proteins. Patients with a homozygous genotype of CYP3A5*3/*3 thus create a nonfunctional enzyme (Kuehl et al., 2001) and end up poorly metabolising carbamazepine.

Our study results showed no statistically significant association of CYP3A5 metaboliser status with the plasma levels of carbamazepine at one, three, six, and 12-month follow-up periods. These results are similar to Panomvana et al. (2013), who studied the effect of CYP3A5 genotypes on carbamazepine levels and clearance in the Thai population. This study was conducted in epileptic patients on carbamazepine monotherapy as well as in patients on carbamazepine with other antiepileptic drugs, such as phenytoin, phenobarbitone, and valproate. They found no significant difference in dose-adjusted carbamazepine levels among expressors and non-expressors. This could be due to the insufficient sample size of 36 patients on carbamazepine monotherapy.

In our study, the number of patients who presented

with ADRs was the same (n=3) in both the study arms. The ADRs occurred in [5 out of 34 (15%)] patients who were CYP3A5 expressors and [1 out of 26 (4%)] patients who were CYP3A5 non-expressors. This finding is in contrast with the study conducted by Ganesapandian et. al. (Ganesapandian et al., 2019) where 16 % of ADRs were found among expressors and 35% of ADRs were found among non-expressors. The clinical carbamazepine toxicity in our study consisted of drowsiness (n=3), giddiness (n = 1), headache (n =1), and weight gain (n = 1). Among these, the patient with giddiness was a CYP3A5 expressor and had plasma levels above the laboratory reference range, and another patient with drowsiness, who was also a CYP3A5 expressor, had plasma levels below the laboratory reference range.

We have conducted this study as a vital step in the combination of pharmacogenetics and TDM to know the determinants of carbamazepine risk minimisation. There are differing results on the effect of the CYP3A5 genotype on carbamazepine levels, and hence, there is a necessity for efficacy guidelines for the genotype-based dosing of carbamazepine along with existing Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for HLA genotype.

Limitations

One of the limitations of our study was the small number of participants. Our study genotyped only CYP3A5, while another isoform may play an important role in the metabolism of carbamazepine.

Conclusion and Recommendations

It is concluded that there is no significant usefulness of the addition of genotyping in risk minimisation of carbamazepine when used with TDM. Genotyping can be considered only in those patients on carbamazepine with symptoms of toxicity. The Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP) technology for genotyping of carbamazepine [as CYP3A5 genotype - expressors or non-expressors] method will eventually be used for routine patient care in a public sector tertiary care teaching hospital in India.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Ethics Committee-1 of Seth G.S. Medical College and K.E.M. Hospital, Mumbai (Date: August 23, 2019, Project Number: EC/OA-41/2019).

Informed Consent:

1. I have read or have had read to me the information given in the Informed Consent Document for this study entitled "A randomized controlled trial to evaluate genotyping and therapeutic drug monitoring vs. only therapeutic drug monitoring as a strategy for risk minimization in epileptic patients on carbamazepine therapy."
2. I have received an explanation of the nature, purpose, duration, and foreseeable effects and risks of the trial and what I will be expected to do. My questions have been answered satisfactorily.
3. I understand that my participation in the study is voluntary and that I may refuse to participate or may withdraw from the study at any time, without penalty or loss of benefits to which I am otherwise entitled.
4. I further understand that any information that becomes available during the course of the study that may affect my willingness to take part will be informed to me.
5. I give permission to allow the study personnel to withdraw my blood (4.0 ml) for the determination of plasma carbamazepine levels and genotyping.
6. Institutional ethics committee authorities may wish to examine my medical records to verify the information collected. By signing/giving a thumb impression on this document, I give permission for this review of my records.
7. I understand that my identity will not be revealed in any report or publication.
8. I agree to take part in the above study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept -MB; Design-MB; Supervision-MB; Resources-MB, VA; Data Collection and/or Processing-MB, SM, SA, HK; Analysis and/or Interpretation-VA; Literature Search-MB, VA; Writing Manuscript-MB, VA, SM, SA, HK; Critical Review-MB; Other-Nil

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Usage of Sonochemistry in Medicine, Industry, Environment, and Synthesis

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ABSTRACT

Much progress has been made regarding ultrasound in chemical science and the chemical industry in recent years. Ultrasonic waves are defined as inaudible sound waves with frequencies exceeding 20 kHz. This paper focuses on using ultrasonic technologies in some areas such as polymer degradation, polymerization reactions, removal of toxic organic contaminants in water, organic synthesis, ultra-strong transfer processes including the extraction process, adsorption process, membrane process, demulsification, crystallization process, emulsification, heterogeneous chemical reaction processes, and the electrochemical process.

Keywords: Environment, industry, medicine, organic synthesis, sonochemistry, ultrasound

Introduction

Much progress has been made regarding ultrasound in chemical science and the chemical industry in recent years. Ultrasonic waves can be defined as “inaudible sound with a high frequency for humans” the frequency of which approximately exceeds 20 kHz. This paper focuses on using ultrasonic technologies in some areas such as polymer degradation, polymerization reactions, removal of toxic organic contaminants in water, organic synthesis, ultra-strong transfer processes including the extraction process, adsorption process, membrane process, demulsification, crystallization process, emulsification, heterogeneous chemical reaction processes, and the electrochemical process.

Sound is a vibration that propagates as an acoustic wave in a transmission medium such as a gas, liquid, or solid. It represents sound waves with wavelengths between 17 meters and 1.7 centimeters in air at atmospheric pressure. Sound waves with frequencies above 20 kHz are called ultrasounds and are inaudible to humans. In contrast, sound waves below 20 Hz are referred to as infrasound. (Figure 1).

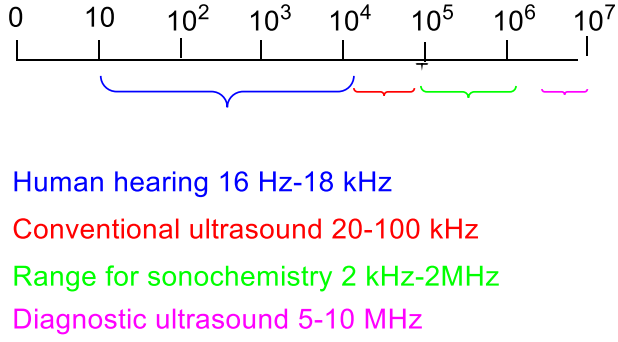

Figure 1. Frequency ranges of sound and its application (Mason and Bernal, 2003)

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Sonochemistry is chemistry assisted by ultrasound. Ultrasound is any sound wave with frequencies above the normal hearing range of the human ear (i.e., above 16 kHz) (Savun-Hekimoğlu, 2020). Ultrasounds have proven useful in many fields, including medical imaging, also called sonography. In addition, it is used to realize and accelerate chemical reactions. Transducers, mostly made of piezoelectric ceramics are used in sonochemistry (Mason, 1997).

As in all chemical reactions, chemical bonds are broken and re-formed in sonochemical processes (Li et al, 2021). When ultrasonic waves (if their frequency is greater than 20 kHz) propagate through a liquid, chemical bonds are broken and free radicals are formed. Ultrasound is used to accelerate organic reactions, increase yield, reduce reaction time, and provide milder reaction conditions. Ultrasound promotes effective mixing in solid-liquid and liquid-liquid mixtures (Kaur, 2019). This feature is also used in various phase transfer-catalyzed reactions (Davidson et al, 1987). The pharmaceutical and food industries (Mason, 1995; Mason et al., 1996; Fresno et al. 2018; Chen et al., 2020; Rastogi et al., 2011; Wang et al., 2018; Sharma et al., 2020), extraction processes (Mason et al., 1996; Ebringerová & Hromádková, 2010; Alves Filho et al., 2020; Juliano et al., 2017; Kumari et al., 2018; Martínez et al., 2020; Ojha et al., 2020; Paniwnyk et al., 2009; Kulkarni & Rathod, 2014; Chen, 2012; Dey & Rathod, 2013; Shoh, 1988; Mason et al., 1990) waste treatment (Shoh, 1988; Ingole & Khedkar, 2012; Savun-Hekimoğlu & Ince, 2017; Gogate et al., 2003; González-Garcíaet al., 2010; Wang & Zhu, 2005; Nejumal et al., 2014; Yousef Tizhoosh et al., 2020; Naddeo et al., 2010; Monsef et al., 2019; Yadav et al., 2020; Serna-Galvis et al., 2019; Zinatloo-Ajabshir et al., 2020; Kerboua et al., 2021; Adewuyi, 2005; Mason & Lorimer, 1988), materials chemistry (Peters, 1996; Yuan et al., 2020; Cruz-Benítez et al. 2021), analytical chemistry (Robin et al., 2017; Wieland et al., 2019; Bendicho, 2012; Seidi & Yamini, 2012), emulsification (Shoh, 1988; Mason & Lorimer, 1988), material processing (Shoh, 1988; Mason & Lorimer, 1988), food processing (Mason et al., 1990; Mason & Lorimer, 1988) and nanoparticle synthesis (Kumari et al., 2018; Monsef et al., 2019; Chatel, 2019; Hujjatul Islam et al., 2019; Dheyab et al., 2021; Ersan, et al., 2020; Asfaram et al.; 2018, Bayrami et al., 2019) are the application areas of ultrasonic waves (Machado et al., 2021).

Applications of Sonochemistry

Based on the studies in the literature, this article will present the studies in the literature in chronological order. Chemical effects from ultrasound in aqueous solution were first described in two separate papers in 1927 (Richards & Loomis, 1927; Wood & Loomis, 1927).

In 1953, Busnel et al. published a paper describing the relationship between wavelength and the oxidation of potassium iodide by ultrasound (Busnel et al., 1953). A similar study was published by Renaud in the same year (Renaud, 1953).

In 1955, an article on the examination of the ultrasonic energy sensor was published by d'Acoustique (d'Acoustique, 1955).

Weissler et al. sonolyzed acetonitrile under argon gas and obtained N_2, CH_4, and H_2 in 1965. When they performed the same experiment under oxygen gas, they obtained N_2, CO, CO_2, and H_2O. The ultrasound pulse-echo method has been used to obtain two-dimensional high-resolution images of the internal structures of the human heart (Åsberg, 1967). Ultrasonic flow detection was used as a useful technique in the evaluation of peripheral vascular disease (Strandness et al., 1967).

Fundamental developments in ultrasound, especially in radiological imaging, began after the 1970s. Leopold and Joel Sokoloff demonstrated that B-scan ultrasonography is useful in cases where radiographic imaging is not available or when hepatobiliary dysfunction is severe enough to preclude oral cholecystography or intravenous cholangiography (1973). Imaging of ventricular septal defects with cardiac ultrasonography has been achieved (King et al. 1973).

Styrene (1) can be cyclopropanated in 96% yield in 1 h when a combination of both sonication and mechanical stirring is used in 1982 (Figure 2) (Regen & Singh, 1982).

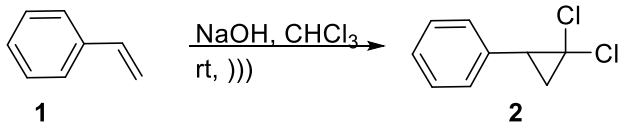

Figure 2. Cyclopropanation of styrene (39)

Davidson performed the N-alkylation of amines, synthesis of ethers and esters, and hydrolysis of esters under ultrasonic conditions in 1987. This study contains results supporting the idea that the presence of oxygen in a cavitating liquid produces singlet oxygen in addition to peroxy radicals. In another study, Henglein discussed the chemical effects of ultrasound used in medical diagnosis (. Henglein, 1987).

In 1988, Mason and Lorimer explained the theory of ultrasound and its application areas in chemistry. Suslick published articles on the chemistry, physical, and biological properties of ultrasound (Suslick, 1988) A year later, Lay and Low described the use of ultrasound in synthesis (1989).

In 1990, Mason published two studies on ultrasound chemistry. Lindley explained the usage of sonochemistry in inorganic and organometallic chemistry including catalysis (1990). Rienz et al. (1990) presented a paper on the sonochemistry of volatile and non-volatile solutes in aqueous solutions.

In 1992, the effects of ultrasonic irradiation at different frequencies, i.e. 20, 40, 60, and 80 kHz, on the activation of a model chemical reaction (iodide to iodine oxidation) were investigated (Cum et al, 1992). Mason et al. (1992) reported how ultrasonic energy input to a chemical reaction is affected by ultrasonic power used, the presence of bubbled gas, temperature, solvent composition, and reaction volume. Ultrasonic diagnostic devices can have possible harmful effects. Riesz and Kondo (1992) discussed the roles of free radicals and the mechanical effects of ultrasound in DNA degradation, inactivation of enzymes, lipid peroxidation, and cell killing.

In 1994, Olson and Barbie presented an advanced oxidation process in which ultrasound was studied in terms of its potential to oxidize natural organic matter to refractory electrolytes. Entezari and Kruus (1994) researched the effect of sonochemistry in the oxidation of iodide.

In 1995, Low explained the use of ultrasound in synthesis. Suslick et al. (1996) described the use of sonochemistry in material synthesis in 1996. In 1996, Ophir et al. presented a paper on ultrasonic imaging of tissue tension and elastic modulus under in vivo conditions in the field of breast imaging. Li et al. (1996) synthesized 5,5-disubstituted hydantoins 4 from ketone 3 $(NH_4)_2CO_3$ and NaCN with ultrasound in 1996 (Figure 3).

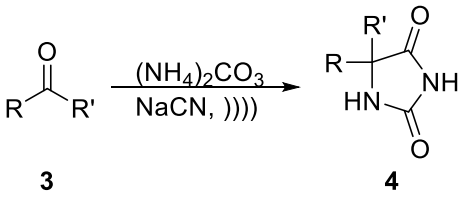

Figure 3. The synthesis of 5,5-Disubstituted Hydantoins 4

The hydrolysis of 4-nitrophenyl ester derivatives 5 (R = Me, Et, Pri, But) was carried out in an ultrasonic bath at 35 °C, and it was observed that the reaction rates increased in 1997 (Mason) (Figure 4).

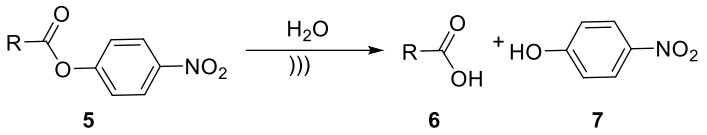

Figure 4. The hydrolysis of 4-nitrophenyl ester derivatives 5

The alkylation reaction of 5-hydroxy-4-oxo-4H-1-benzopyran-2-carboxylic acid ethyl ester (8) was carried out by Mason using several different haloalkanes under sonication in 1997.

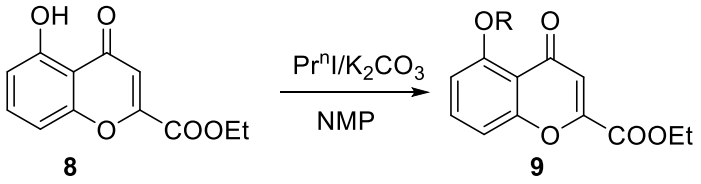

Figure 5. The alkylation reaction of 5-hydroxy-4-oxo-4H-1-benzopyran-2-carboxylic acid ethyl ester (8)

In the same study, he used ultrasound to increase the reactivity of the metal and saw that the reaction rate increased as a result (Figure 5). In another a study Petrier explained the degradation rate of phenol and carbon tetrachloride at different frequencies, 20 kHz, 200 kHz, 500 kHz, and 800 kHz (1997).

Fillion and Luche (1998) described the effect of sonochemistry in cycloaddition reactions in their books. Crum et al. (1999) published a chapter on sonochemistry. Lubinski et al. (1999) described the use of ultrasound in medical ultrasound elasticity imaging.

There are examples of sonochemical [4+2] and [3+2] cycloaddition reactions in the literature. One of these reactions is the addition of o-benzoquinone (10) with furan. The effect of sonochemistry has been investigated in studies (Figure 6) (Cabello et al, 2003; Avalos et al., 2003).

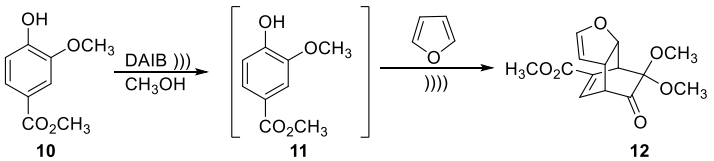

Figure 6. Sonochemical [4+2] and [3+2] cycloaddition

reactions

Li et al. (2003) succeeded in the synthesis a,a-bis(substituted benzylidene) cycloalkanones 14 catalyzed by KF/Al_2O_3 under ultrasound irradiation (Figure 7).

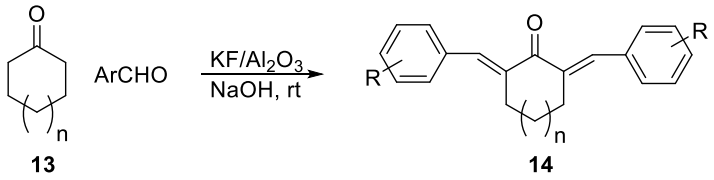

Figure 7. The synthesis of substituted benzylidene cycloalkanones

Leveque and Cravotto (2006) explained a method using ultrasound in green chemistry. For example, Hydrazinolysis of methyl salicylate 15 occurred in ultrasound with a yield of 79%. Another example is the Mannich reaction of 17 under ultrasound irradiation (Figure 8).

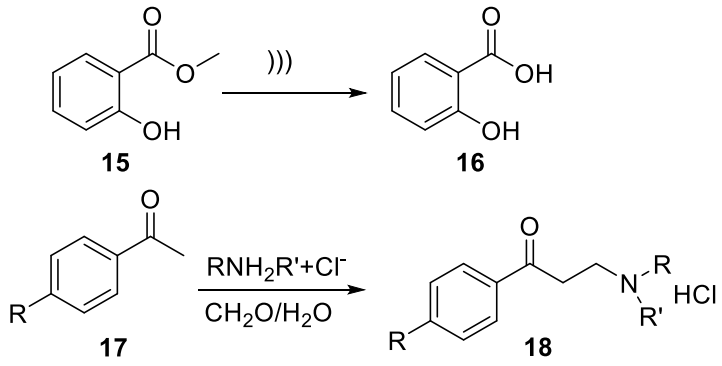

Figure 8. Reaction examples in ultrasound irradiation

Guzen et al. (2006) explained that the condensation reactions of o-phenylenediamines with a diketone or a series of ketones occurred by ultrasound irradiation in the presence of APTS with good to excellent yields without catalyst (Figure 9).

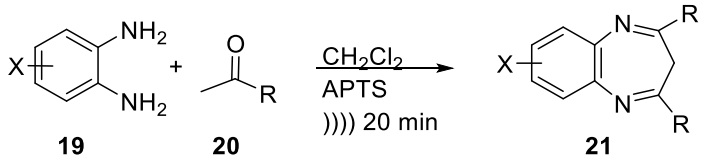

Figure 9. The synthesis of benzodiazepines 21

Hickenboth et al. succeeded in the synthesis of molecule 24 by sonication (Figure 10) (2007).

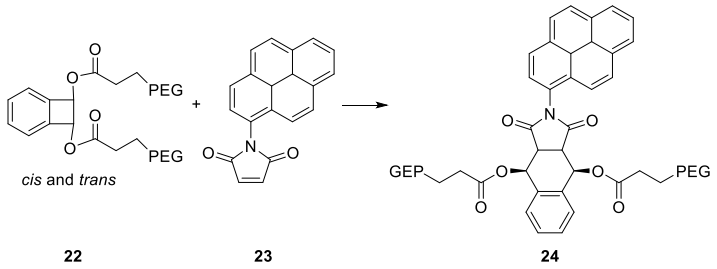

Figure 10. Ring-opening reaction of benzocyclobutene mechanophore (22)

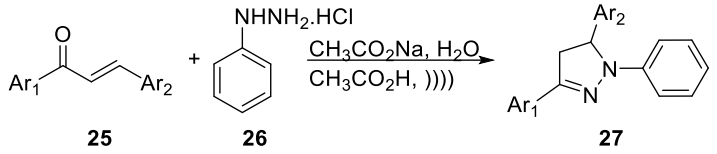

Figure 11. Synthesis of Pyrazoline Derivatives

1,3,5-triaryl-2-pyrazolines 27 were synthesized with chalcones 25 and phenylhydrazine hydrochloride 26 under ultrasound irradiation in 83-96% yields (Figure 11) (Li et al., 2007).

Anna et al. (2009) synthesized 2-substituted-2-imidazolines 30 from the substituted aldehydes and ethylenediamine by ultrasound irradiation with NBS in an aqueous medium (Figure 12).

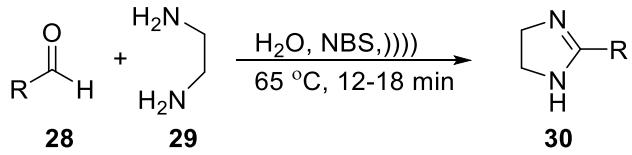

Figure 12. Synthesis of 2-Imidazolines (30)

2,4,5-Triarylimidazoles 32 were synthesized by the one-pot three-component condensation of benzil/benzoin 31, aldehydes, and ammonium acetate in the presence of the catalytic amount of ceric (IV) ammonium nitrate under ultrasound (Figure 13) (Shelke, et al., 2009).

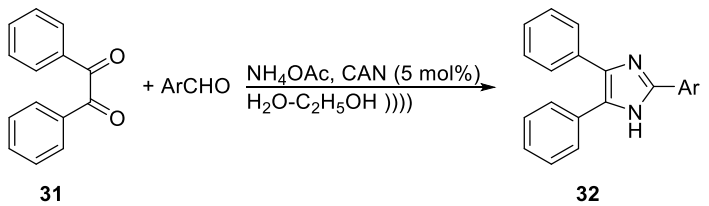

Figure 13. Synthesis of 2,4,5-triarylimidazole derivatives (32)

Jin et al. (2009) studied the epoxidation of chalcones 33

with urea-hydrogen peroxide (UHP) in an ultrasonic bath and obtained epoxide 34 with high yields (Figure 14).

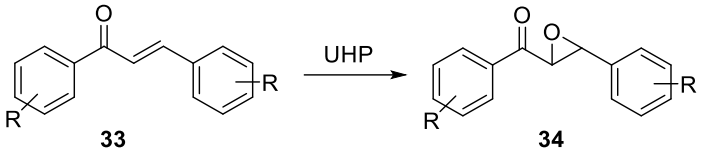

Figure 14. The epoxidation of chalcones

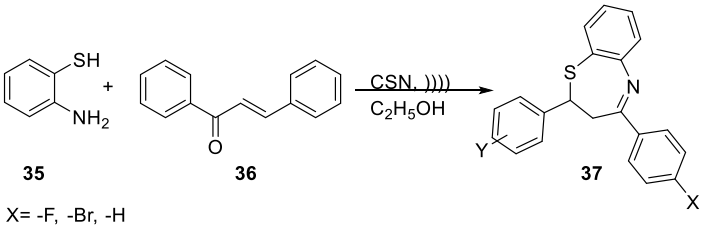

Figure 15. Synthesis of 1,5-benzothiazepines 37 from chalcones 36 and o-aminothiophenol 35

Chate et al. (2011) found an easy method for the preparation of 1,5-benzothiazepine derivatives in the presence of 10 mol% catalysts of CAN under ultrasonic irradiation (Figure 15).

Ultrasound is used to destroy toxic organic molecules using advanced oxidation processes (AOPs). Thus, it can control pollution and environmental protection. Bremner et al. (2011) used ultrasound at different frequencies for the oxidation of polycyclic aromatic hydrocarbons and phenol and examined the effect of ultrasound on degradation.

Zou et al. (2011) developed a method for preparing dihydropyrano[2,3-c]pyrazoles via a four-component reaction of aromatic aldehydes, hydrazine, ethyl acetoacetate, and malononitrile under ultrasound irradiation in water.

The Michael addition reaction of some active methylene compounds 39 to cyclohexenone 38 was achieved in high yields under ultrasonic irradiation, without using solvents (Figure 16) (Oge et al., 2012).

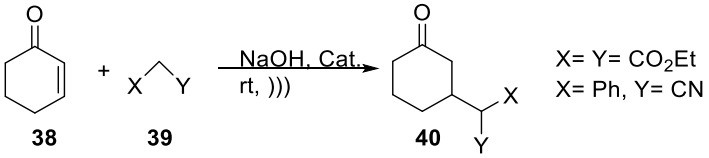

Figure 16. The Michael addition reaction to cyclohexenone 38

IKK-b inhibitors, A2A adenosine receptor antagonists and potent HIV-1 integrase inhibitors (Safari et al., 2012) and 2-amino-pyridine derivatives 44 identified as anti-tuberculosis agents (Chunxia 2013), are potential derivatives for the treatment of prion disease (Figure 17) (Guo et al., 2008).

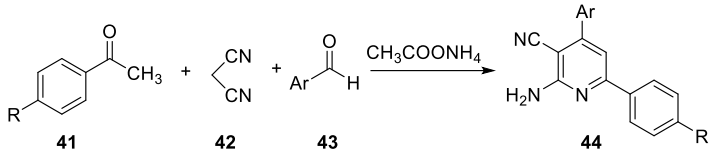

Figure 17. The synthesis of 2-amino-pyridine derivative 44

Eftekhari-Sis and Vahdati-Khajeh (2013) published a three-component, catalyst-free swift, and efficient procedure for the synthesis of pyridazine (26) and pyrrole (29) derivatives using water as a solvent and under ultrasonic irradiation (Figure 18).

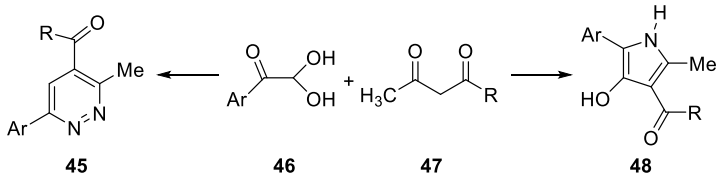

Figure 18. The synthesis of pyridazine 45 and pyrrole 48 derivatives

Mady et al. (2013) carried out the synthesis of homoallylic alcohols (50, 51) with ultrasonic support in high yields (Figure 19).

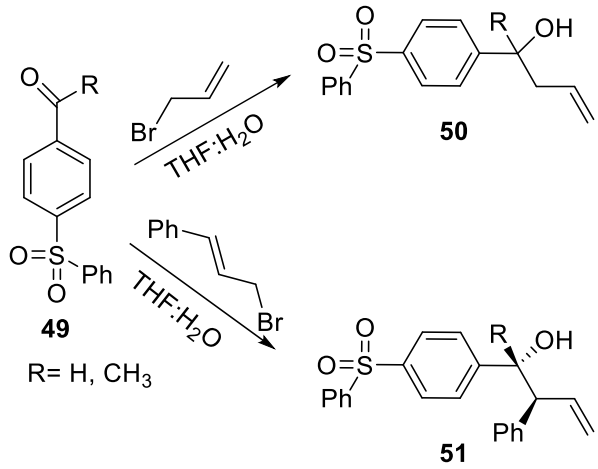

Figure 19. The synthesis of homoallylic alcohols (50, 51)

Cravotto et al. (2015) tried a series of reactions such as oxidation, bromination, aza-Michael, C-C couplings, MCR,

and aldol reactions in water under sonochemical conditions, thus showing that organic synthesis can be successful under sonochemical conditions in green chemistry.

Silva et al. (2016) explained the preparation of isatin derivatives 53 using 5-azido-spiro[1,3-dioxolane-2,3'-indol]-2'(1'H)-one (52) in the presence of various alkynes under acidic conditions and ultrasound irradiation (Figure 20).

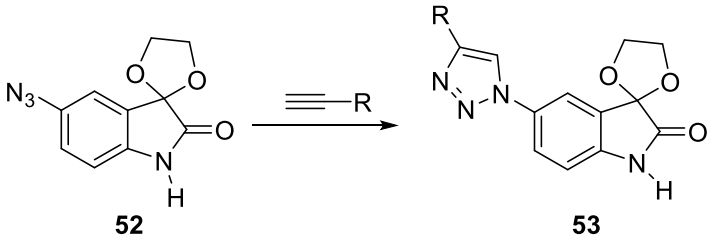

Figure 20. The synthesis of isatin derivatives 53

Zhang (2016) explained that sonochemistry can be used in the chemical and chemical industries in 2016. Draye et al presented a review of the recent improvements in sonochemical and combined sonochemical oxidation processes.

Crawford succeeded in the synthesis of molecules 54 and 55 in an ultrasound bath with high yields in 2017 (Figure 21).

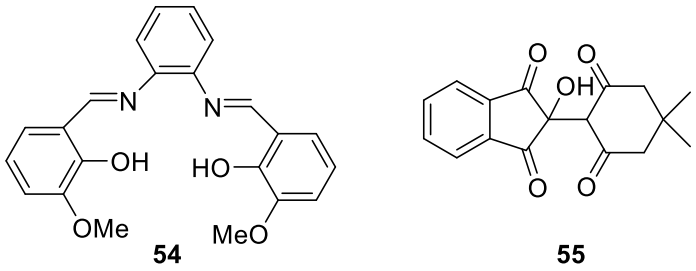

Figure 21. The synthesisis of diamine 54 and aldol product 55

Sharda et al. (2018) also studied the condensation reactions of o-phenylenediamines 56 and synthesized 2,3-dihydro-1H-1,5-benzodiazepines 58 using a catalyst in 2018 (Figure 22).

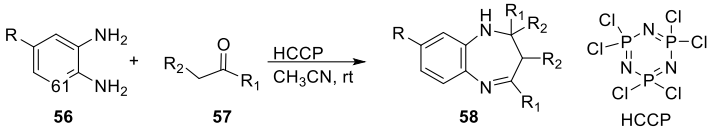

Figure 22. The condensation reactions of o-phenylenediamines 56

Navjeet explained the synthesis of nitrogen containing five-membered heterocycles under ultrasonic ırradiation in 2019.

In 2020, Mallakpour and Azadi presented a sonochemical method for organo-synthesis, organo-modifications, and preparation of hybrids of titanium dioxide (TiO_2), which can be used in a wide variety of fields such as photocatalysis, antibacterial agents, present, self-cleaning.

Gharat et al. (2020) studied the application of sonochemistry in commercial industries such as food, pharmaceuticals, cosmetics, and chemicals and designed the reactor accordingly and stated that the ultrasonic system is more suitable in terms of cost-effectiveness, high efficiency, low waste, and low energy (2020). Sonochemistry is also used successfully in polymer synthesis. Hatami et al. are interested in ultrasound-assisted emulsion polymerization, mass and suspension polymerization, ring-opening polymerization, preparation of hydrogel polymers, synthesis of copolymers, phase transfer catalysis polymerization, reversible addition-fragmentation chain transfer (RAFT) procedure, polymerization (ATRP) and preparation of polymer nanocomposites were accomplished under ultrasonic conditions (2020). Metal-supported work can be done with ultrasonication. Since ultrasonic irradiation affects the metal surface, it also changes the mechanism, speed, and duration of the reaction. Bhuyan et al. tried ultrasonic conditions for various coupling reactions and were successful (Figure 23).

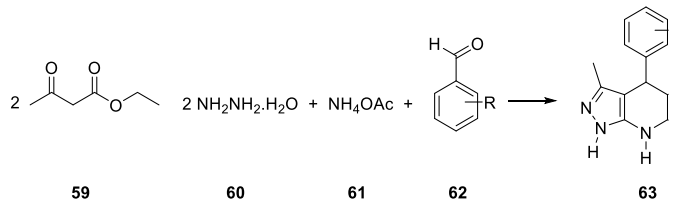

Figure 23. The synthesis of pyrazole 63

Tran and Nguyen have worked on alkylation reactions and used sonochemistry reactions, an environmentally friendly method, to improve reaction rates, product yield,

and selectivity and to find new synthetic routes in 2020. Blanco et al. (2020) synthesized 1,3-dibenzyl-1H-4,5,6,7-tetrahydro-1,3-diazepinium and 1,4,5,6,7,8-hexahydro-1,3-diazepinium salts under ultrasonic conditions with high yields (Figure 24).

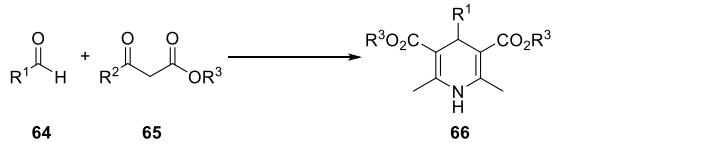

Figure 24. The synthesis of diazepinium 66

Draye et al. (2020) presented a review on the application of ultrasound in green chemistry in 2020. In 2021, the semisynthetic reaction of enantiopure Milbemycin-β (68) is successful with sonication in the first step but fails without sonication (Figure 25).

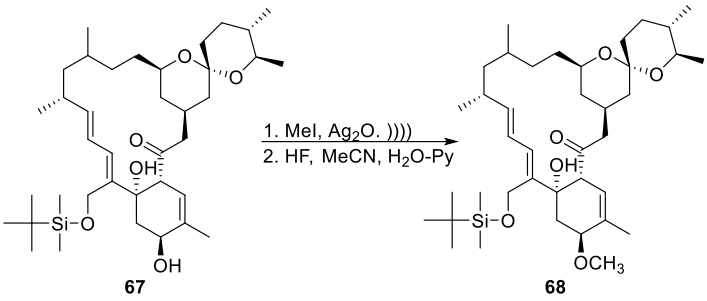

Figure 25. The synthesis Milbemycin-β (68)

Machado explained the synthesis of N-heterocyclic compounds 69 and 70 that are pharmacologically important using green solvents and catalysts in the ultrasonic bath in 2021 (Figure 26).

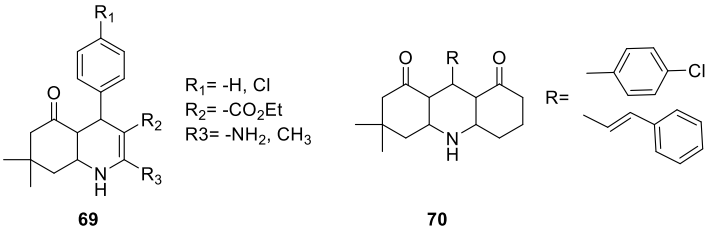

Figure 26. The synthesis of N-heterocyclic compounds

Casey et al. (2022) have written a review on the use of ultrasound to measure skeletal muscle. Shabir et al. (2022) presented the synthesis of 5-7 membered heterocyclic rings in sonochemistry with high yields.

Kamble et al. (2022) developed an environmentally friendly synthesis method for bioactive molecules (71-74) containing nitrogen and sulfur using ultrasonic energy in a short time (Figure 27).

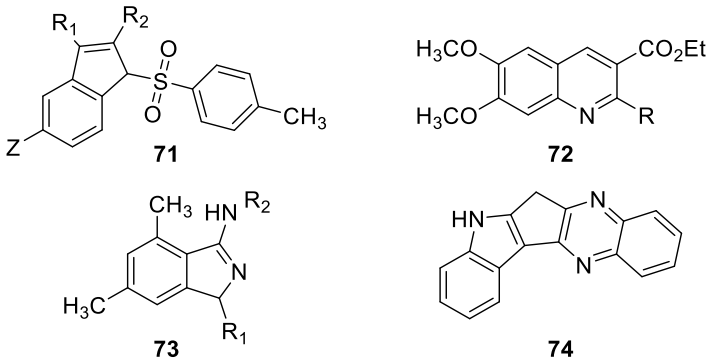

Figure 27. Synthesiszed bioactive molecules 71-74 using ultrasonic energy

In 2023 Sezen et al. studied the addition reaction of dimethyl cyclohexa-1,4-diene-1,2-dicarboxylate (46) and dichloroketene under ultrasonic and non-ultrasonic conditions. They showed that the ultrasonic reaction is faster than the non-ultrasonic reaction (Figure 28).

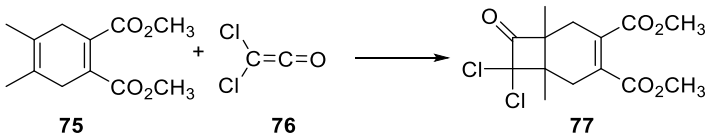

Figure 28. The ketene addition reaction of dimethyl cyclohexa-1,4-diene-1,2-dicarboxylate 77

Barmin et al. (2023) explained new perspectives on ultrasound-responsive polymeric designs, envisaging their current and future applications in ultrasound imaging and therapy. Abdelmonsef et al. (2023) found that ultrasound-assisted green synthesis of triazole analogs is a promising class of molecular entities for the development of new anticancer therapies, through targeting of some Rab proteins (Figure 29).

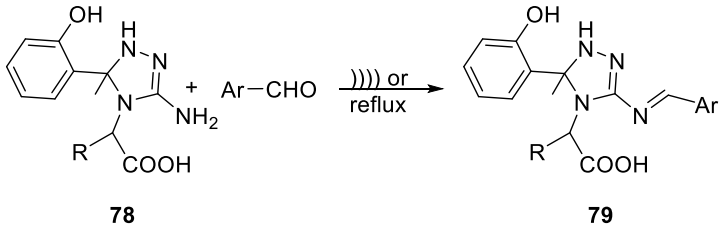

Figure 29. The synthesis of triazole analogs

In 2024, Jiao et al. (2024) wrote a review on ultrasound-

assisted sonochemical synthesis and biological applications, to promote the next generation of ultrasound technology-assisted applications.

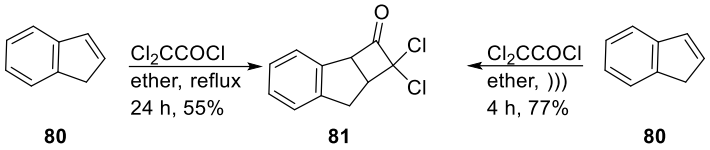

Figure 29. The synthesis of dichloro ketone 81.

Gökçay Bilici et al. (2024) synthesized dichloro ketone 81 with the addition reaction dichloroketene to indene with high yields under ultrasonic conditions.

Conclusion

The article gives short but detailed information about sonochemistry with various applications in the medicinal-pharmaceutical-drug-chemistry industries, and imaging techniques. While information about sonochemistry is given in the article, developments, and syntheses year by year since 1927 are mentioned. Much progress has been made regarding ultrasound in chemical science and the chemical industry in recent years. This paper focuses on using ultrasonic technologies in some areas such as polymer degradation, polymerization reactions, removal of toxic organic contaminants in water, organic synthesis, ultra-strong transfer processes including the extraction process, adsorption process, membrane process, demulsification, crystallization process, emulsification, heterogeneous chemical reaction process, and electrochemical process.

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