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Aim & Scope

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The Journal particularly aims to foster the dissemination of scientific information by publishing manuscripts related to current Pharmaceutical as well as Allied sciences fields. IJPRAS publishes full research papers presenting original, high quality research, critical review articles providing comprehensive analysis of research & development within a defined area.

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Aim

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Contents

Anti-inflammatory Activity of Two Antitussive Plants for Children: Sericanthe chevalieri and Ceiba pentandra

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ABSTRACT

The objective of this study was to document the plants used in traditional cough treatment for children and to conduct a phytochemical study of the two most used plants. An ethnobotanical survey was conducted among traditional sellers in the markets of Bamako. A phytochemical screening based on classic color reaction and tube precipitation methods was performed. Aluminum trichloride was used to quantify flavonoids, whereas the FolinCiocalteu method was utilized to quantify total polyphenols. The anti-protein denaturation method was used to assess the extracts' anti-inflammatory properties. In total, 56 participants, including 42 women and 14 men, were interviewed. The survey inventoried 17 antitussive plant species belonging to 14 botanical families. Sericanthe chevalieri (S. chevalieri) and Ceiba pentandra (C. pentandra) were the most frequently cited species. Phytochemical screening of these two plants revealed the presence of many major chemical groups such as alkaloids, terpenes, coumarins, tannins, saponins, and flavonoids. Macerated extracts (hydroethanolic and aqueous) exhibited the highest levels of phenolic and flavonoid compounds in both plants. The best antiinflammatory potential, indicated by the degree of anti-protein denaturation, was observed in the hydroethanolic extracts, with an IC50 of 263.48 ± 20.80 µg/mL for S. chevalieri and 420.30 ± 19.80 µg/mL for C. pentandra. This study demonstrates that the extracts of C. pentandra and S. chevalieri are rich in bioactive substances with significant anti-inflammatory potential, which may confer antitussive properties.

Keywords: Sericanthe chevalieri, Ceiba pentandra, Ethnobotanical survey, Phytochemical screening, Anti inflammatory activity, Cough

INTRODUCTION

Cough is a natural and essential reflex that helps the body clear the respiratory airways [1-3]. It is the main symptom of respiratory infections or non-infectious conditions, often affecting children under the age of five. Most commonly, cough is associated with cold symptoms, particularly nasal congestion. However, it can also be triggered by other causes such as gastroesophageal reflux (GERD), bronchial inflammation (asthma, allergy), or environmental factors like tobacco smoke. In 2021, according to pediatricians, cough and cold symptoms accounted for 60% of medical consultations in Mali [4].

Antitussives used for treating cough include expectorants (guaifenesin, erdosteine, hypertonic saline solution) and agents that reduce the viscosity of secretions, such as mucolytics and mannitol [1, 5, 6]. However, alerts from the World Health Organization (WHO) regarding the safety of cough syrups, such as the 2022 case in Gambia where 66 children died, highlight the need for safer alternatives [7]. In this context, the use of medicinal plants presents an interesting and hopeful prospect [8-12].

Plants are known for their intense metabolic activity, resulting in the synthesis of a wide variety of bioactive compounds [13-18]. Many medicinal plants possess expectorant and mucolytic properties, and their use is welldocumented in various cultures [19-22]. When well-studied and properly used, traditional remedies may provide an effective and safer alternative to conventional treatments. These remedies typically have fewer side effects, as they are often consumed in more natural and less processed forms. Moreover, medicinal plants are more accessible and affordable, making them especially attractive to populations in developing countries [13, 18, 23]. Additionally, these plants can not only alleviate symptoms but may also target the underlying causes of illness due to their diverse bioactive compounds [24]. It is within this context that the present study was undertaken to document the medicinal plants used in treating cough in children in Bamako, Mali. Biological investigations were conducted on the plants most recommended by traditional healers.

MATERIALS AND METHODS

Our study initially involved conducting an ethnobotanical survey among traditional healers, vendors, medical doctors, and research professors. The goal was to select plants based on citations, to carry out experimental studies in the Laboratory of Food Biochemistry and Natural Substances (LBASNa), Faculty of Sciences and Techniques (FST), at the University of Sciences, Techniques, and Technologies of Bamako (USTTB).

Zone of investigation

The survey was conducted in Bamako, specifically in the neighborhoods and markets of Lafiabougou, Hamdallaye ACI, Djikoroni ACI, and Kalaban Coura ACI. Figure 1 provides a map of the investigation areas.

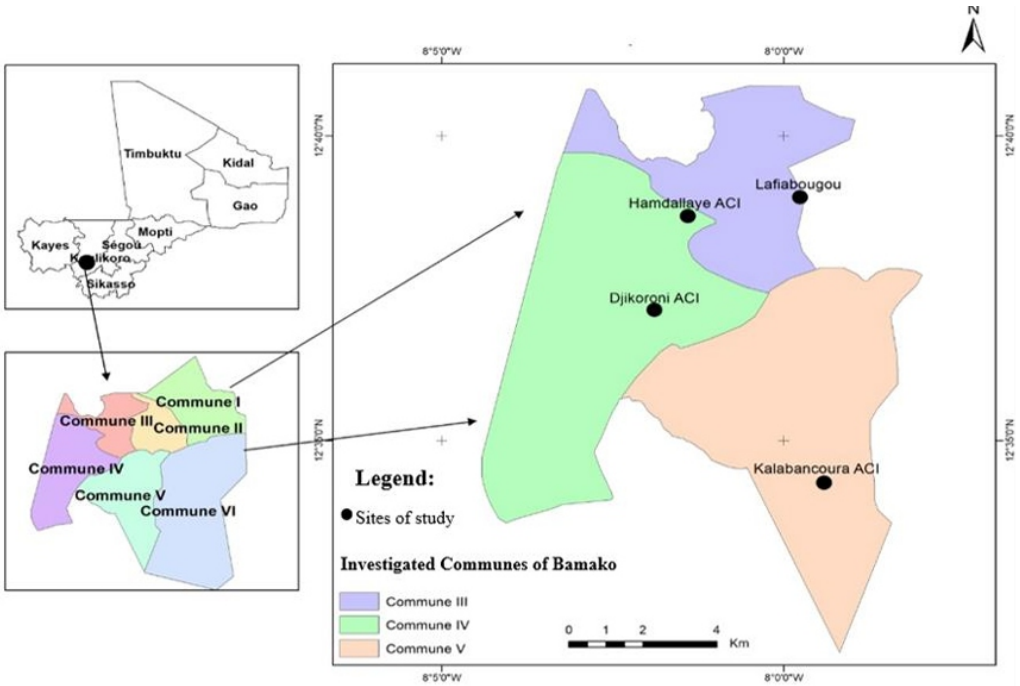

Figure 1. Survey area map showing four neighborhoods of Bamako, Mali.

Plant material

The plant material consisted of the bark of Ceiba pentandra (L.) Gaertn. and the leafy branches of Sericanthe chevalieri (K. Krause) Robbr., which were purchased from markets in Bamako. The identification of the studied plants was carried out at the Laboratory of Botany and Ecotoxicology of the Faculty of Sciences and Techniques (FST) at the University of Sciences, Techniques, and Technologies of Bamako (USTTB). The samples were carefully washed, dried at room temperature, then ground into powder and stored away from light and moisture.

Methods

Ethnobotanical survey

A survey form was used to document the plant species employed for treating cough in children, based on interviews conducted with market vendors and residents of the survey areas in Bamako for six months (from February to July 2022).

Study population

The study population included market vendors, traditional healers, research professors, medical doctors, and herbalists. These participants were randomly selected. The purpose of the study was explained to them to obtain their verbal or written free consent.

Preparation of extracts

Maceration was performed with 50 g of powdered plant material in 1500 mL of distilled water or 70% ethanol for 24 hours at room temperature (25–30 °C), followed by vacuum filtration. The decoction was carried out with 50 g of powdered plant material in 1500 mL of distilled water, boiled for 15 minutes at room temperature, and then filtered under vacuum.

Characterization of phytochemicals

Qualitative tube reactions were performed with the obtained extracts to identify the following phytochemical groups: polyphenols (including tannins, flavonoids, saponins, and coumarins), alkaloids, and terpenes, following the protocol described by Konaré et al. [14, 25]. The results were evaluated as follows: positive (+) and negative (-).

Total phenolic compounds assay

The quantification of phenolic compounds was performed using the Folin-Ciocalteu method, following a protocol described by Konaré et al. [14]. 500 µL of Folin-Ciocalteu reagent (diluted to 10% in distilled water) was added to 100 µL of the extract, followed by 400 µL of Sodium Carbonate (Na2CO3) at 75 mg/mL. The reaction mixture was incubated for 2 hours at room temperature, protected from light, and the absorbance was measured at 765 nm. Using the same process, a calibration curve was created using gallic acid at different concentrations (0–100 µg/mL). The calibration curve's regression line was used to calculate the amounts of phenolic chemicals present in the extracts. The results are given in milligrams of gallic acid equivalent per gram of extract (mg GAE/g). Every measurement was done three times.

Determination of total flavonoids

The estimation of total flavonoids was carried out using the aluminum chloride method, following the protocol described by Konaré et al. [14]. 200 µL of extract, 800 µL of distilled water, and 50 µL of a 5% sodium nitrite ($NaNO_2$) solution were added, respectively. After 5 min of incubation at room temperature

(25–30 °C), 50 µL of 10% (w/v) AlCl3 was added to the mixture. After re-incubation of 6 min, 400 µL of 1 M sodium acetate, and 1 mL of distilled water were also added. After homogenization, the absorbance was read at 510 nm using a spectrophotometer (Thermo Scientific, Biomate 3S). A calibration curve was obtained under the same operating conditions with quercetin at different concentrations from 20-120 µg/mL. The levels of flavonoids were deduced by linear regression equation obtained from the calibration curve and then expressed in milligrams of quercetin equivalent per gram of extract (mg QE/g).

Anti-inflammatory activity

The process of denaturing proteins was done according to Gambhire et al. [26] instructions, updated by Koné et al. [27]. To get final concentrations of 62.5, 125, 250, 500, and 1000 µg/mL, the reaction mixture (5 mL) was composed of 1 mL of egg white solution, 3 mL of phosphate-buffered saline (PBS, pH 6.4), and 1 mL of extracts at different concentrations. As the control, the same volume of distilled water was used. The mixtures were heated to 70 °C for five minutes after being incubated for fifteen minutes at 37 °C. At 660 nm, the absorbance was measured following cooling. To determine absorbance and viscosity, sodium diclofenac was utilized as a reference molecule and treated similarly at final concentrations of 62.5, 125, 250, 500, and 1000 µg/mL. The percentage inhibition of protein denaturation was calculated using the method described by Koné et al. [27], with the equation provided below:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Absorbances of samples}}{\text{Absorbances of control}}\right) \times 100$$

Data analysis

The survey data were analyzed using SPSS software. For the statistical processing of quantitative variables (total phenolic content, total flavonoid content, and anti-inflammatory activity), Minitab v18.1 software was employed. Fisher's test with a significance level of P = 0.05 was applied in conjunction with analysis of variance (ANOVA) to compare the mean values of these variables.

RESULTS AND DISCUSSION

Ethnobotanical survey

A total of 56 people, including 42 women and 14 men, were interviewed. The respondents were aged between 25 and 75 years, with the majority being between 25 and 35 age group. The surveyed population included traditional practitioners (the majority), vendors, researchers, physicians, and herbalists. From the answers, the primary mode of administration for treating cough in children was oral, while decoction was the most common extraction method, with 86% of citations. The plants cited and their frequencies of citation are presented in **Table 1.**

Table 1. List and fidelity level of recommended plants

Scientific names	Botanical families	Organs used	Fidelity Level (FL) (%)
Grossopteryx febrifuga	Rubiaceae	Leaves + Seeds	75.00
Anacardium occidentale	Anacardiaceae	Branches	23.21
Ceiba pentandra	**Bombacaceae**	**Bark**	**19.64**
Sericanthe chevalieri	**Rubiaceae**	**Leaves**	**19.64**

Pteleopsis suberosa	Combretaceae	Branches	17.86
Acacia albida	Fabacea	Bark	12.50
Mangifera indica	Anacardiaceae	Leaves	5.36
Vitex mandiensis	Lamiaceae	Leaves	3.57
Piliostigma thonningii	Fabaceae	Leaves	1.79
Acacia nilotica	Fabaceae	Leaves + Seeds	1.79
Ximenia amercanalim	Olacaceae	Branches	1.79
Vitellaria paradoxa	Sapotaceae	Leaves	1.79
Saba senegalesis	Apocynaceae	Leaves	1.79
Guiera senegalesis	Guiera	Leaves	1.79
Pterocarpus erinaceus	Fabaceae	Leaves	1.79
Ficus thonningi	Moraceae	Leaves	1.79

Fidelity level or index (LF) is the percentage of informants who cited the use of a given species in the treatment of a pathology.

A total of 17 species were recorded. The Rubiaceae family was the most represented, followed by the Anacardiaceae family. S. chevalieri and C. pentandra were the most cited, each with a fidelity level (FL) of 19.64%. As a result, these species were selected for biochemical and biological characterizations.

Phytochemical study

The outcomes of the maceration extracts' phytochemical screening (aqueous and hydroethanolic) and the decoction of S. chevalieri and C. pentandra are summarized in **Table 2.**

Table 2. Phytochemical composition of S. chevalieri and C. pentandra extracts

	S. chevalieri			*C. pentandra*		
phytochemical groups	**Aqueous maceration**	**Hydroethanol ic maceration**	**Decoction**	**Aqueous maceration**	**Hydroethanolic ic maceration**	**Decoction**
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Saponines	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+

(+): presence et (-): absence

Table 2 highlighted the presence of various phytoconstituents such as flavonoids, tannins, coumarins, terpenoids, and alkaloids in the extracts of the sampled plants. However, saponins were absent in the decoction extracts of both plants.

Polyphenol and total flavonoid contents

The levels of specified polyphenols and flavonoids are shown in **Table 3.**

Table 3. Polyphenol and total flavonoid contents of S. chevalieri and C. pentandra extracts

Plants	Extracts	Flavonoids (mg QE/100g)	Polyphenols (mg GAE/g)
S. chevalieri	Aqueous macerate	0.268 ± 0.005^{aB}	0.305 ± 0.005^{cA}
	Hydro-ethanol macerate	0.125 ± 0.005^{cB}	0.648 ± 0.022^{aA}
	Decocted	0.159 ± 0.002^{bB}	0.431 ± 0.014^{bA}
C. pentandra	Aqueous macerate	0.468 ± 0.010^{aA}	0.146 ± 0.019^{cB}
	Hydro-ethanol macerate	0.070 ± 0.002^{cA}	0.544 ± 0.024^{bA}
	Decocted	0.357 ± 0.008^{bA}	0.350 ± 0.025^{bB}

*Means that do not share any lowercase letters differ greatly for each plant. The means for each extract that does not share any capital letters are notably different.

The analysis of the assays revealed that the flavonoid concentrations were higher in the aqueous extracts for both plants S. chevalieri and C. pentandra), with values of 0.268 ± 0.005 and 0.468 ± 0.010 mg QE/100 g, respectively. In contrast, the polyphenol levels were higher in the hydroethanolic maceration extracts, with values of 0.648 ± 0.022 and 0.544 ± 0.024 mg GAE/g for S. chevalieri and C. pentandra, respectively.

Anti-inflammatory activity

The results of the protein denaturation inhibition capacity with S. chevalieri and C. pentandra extracts are presented in **Figures 2 and 3.**

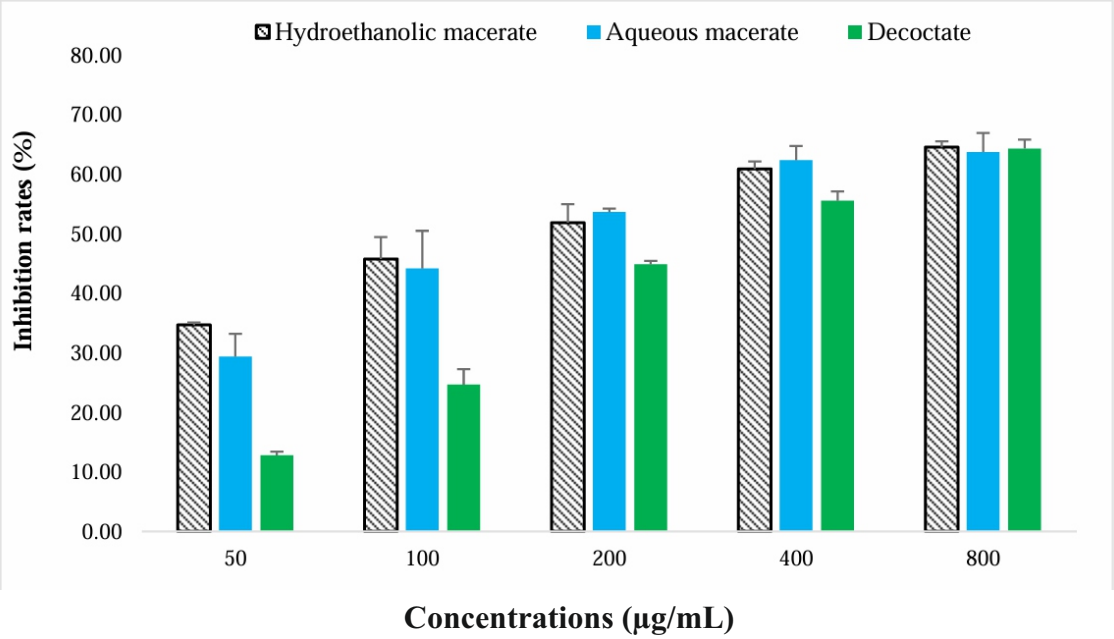

Figure 2. Effects of S. chevalieri extracts on protein denaturation

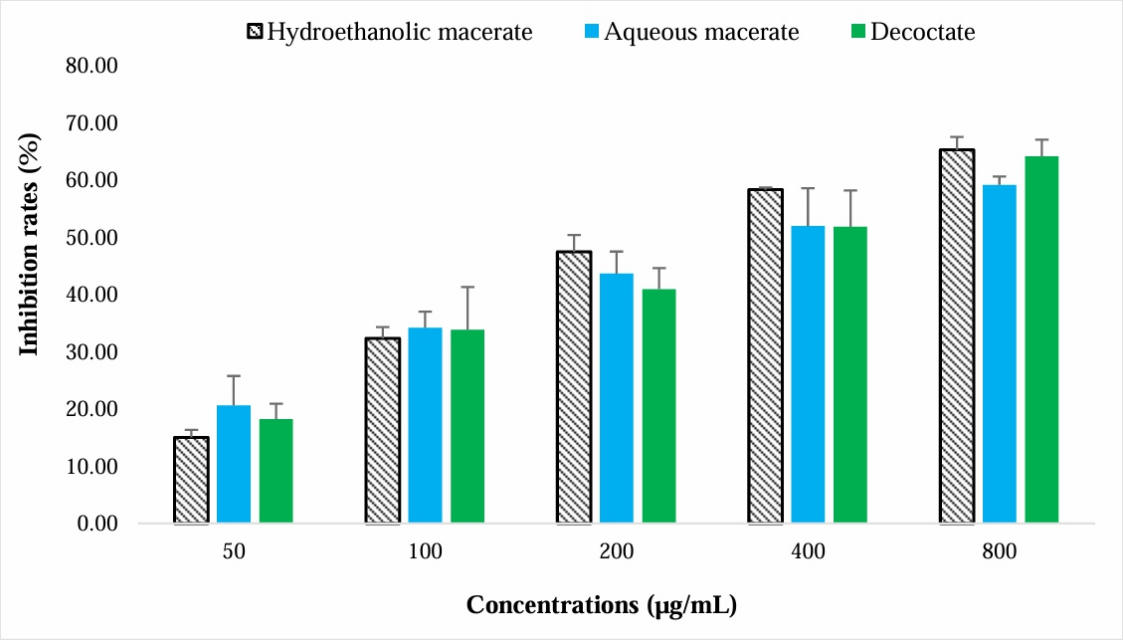

Figure 3. Effects of C. pentandra extracts on protein denaturation

Figures 2 and 3 demonstrated that extracts of S. chevalieri and C. pentandra could inhibit protein denaturation at concentrations ranging from 50 to 800 µg/mL.

The inhibition rates of 50% protein denaturation (IC50) by extracts of S. chevalieri and C. pentandra are shown in **Table 4.**

Table 4. Concentrations of extracts inhibiting 50% protein (Ic50)

	IC_{50} (µg/mL)	
Extracts	***C. pentadra***	***S. chevalieri***
Aqueous macerate	485.60 ± 0.00^{a}	292.48 ± 13.97^{c}
Hydroethanolic macerate	420.30 ± 19.80^{b}	263.48 ± 20.80^{b}
Decocted	464.00 ± 18.30^{a}	429.12 ± 14.47^{a}
p-value	0.006 < 0.05	0,00004 < 0.05

**For each plant species, means that do not share any letters are significantly different.*

The aqueous and hydroethanolic extracts of S. chevalieri exhibited the highest inhibition capacities, with Ic50 values of 292.48 ± 14 µg/mL and 263.79 ± 20 µg/mL, respectively. The extracts of C. pentandra did not show significant differences depending on the extraction method, with IC50 values of 485.62 µg/mL, 420.26 ± 20 µg/mL, and 463.98 ± 14 µg/mL for aqueous, hydroethanolic, and decoction extracts, respectively **(Table 4).**

Cough in children is a recurring health issue that concerns many parents. Given the risks associated with conventional medications, particularly for children, it is crucial to explore safer, cost-effective, and sustainable alternatives. In this context, the use of medicinal plants presents a promising alternative. This study aimed to catalog local medicinal plants used for treating coughs in children in Bamako. Among those surveyed, 42% were women and 12% were men. The survey identified 17 plant species

across 14 families, with C. pentandra and S. chevalieri being the most notable, each with a fidelity index (NF) of 19.64%. The most represented families were Rubiaceae, Anacardiaceae, and Bombacaceae. The most used plant parts were leaves with 72 citations, followed by branches and bark with 35 and 12 citations, respectively. This preference for leaves may be attributed to their role as storage sites for secondary metabolites responsible for the medicinal properties of these plants [28-31]. The use of leaves is encouraged as it poses minimal risk to plant regeneration and contributes to the conservation of floral biodiversity [32]. The survey revealed that the most used mode of administration is oral. Romuald et al. [32] suggested that this preference for oral administration may be attributed to the fact that many of the treated conditions are associated with bacterial and fungal infections located in deeper organs [32]. Additional studies on the pharmacological properties of C. pentandra have shown that both leaves and bark are used to treat various pathologies, including cough and fever [20, 21, 33-38].

The phytochemical screening in this investigation showed that while saponins were missing from the decoctions of both plants, secondary metabolites such as alkaloids, tannins, flavonoids, coumarins, and terpenoids were present in the different extracts. These results differ from those reported by Tala et al. [38], which showed the presence of saponins in the bark of C. pentandra. This discrepancy could be attributed to methodological differences or climatic variations [39-42].

The analysis of the assays revealed higher flavonoid content in the aqueous extracts of S. chevalieri and C. pentandra (0.268 ± 0.005 and 0.468 ± 0.010 mg QE/100 g, respectively). The polyphenol content was higher in the hydroethanolic macerations (0.648 ± 0.022 and 0.544 ± 0.024 mg GAE/g for S. chevalieri and C. pentandra, respectively). This suggests that hydroethanolic and aqueous macerations are the most effective extraction methods for polyphenols and flavonoids, respectively. These findings are in line with the literature reporting the effectiveness of this solvent phenolic compounds extraction and biological activity [25, 31, 43, 44]. This richness in polyphenols and flavonoids may be responsible for their antioxidant activities and could explain their traditional use in treating inflammatory diseases [17, 21, 45, 46]. Indeed, Loganayaki et al. [36] demonstrated that extracts of C. pentandra contain high levels of polyphenols and flavonoids, which are correlated with their antioxidant properties.

Protein denaturation is one of the primary causes of inflammation [25, 26, 47]. This parameter is used to evaluate the anti-inflammatory activity of the extracts [21, 46]. Figure 2 showed that the anti-inflammatory activity was higher in the aqueous extracts of S. chevalieri. For C. pentandra, the anti-denaturation activity was similar across all extracts (Figure 3). However, the anti-inflammatory activity of the extracts from both species was lower compared to that of diclofenac. Table 4 showed that the aqueous and hydroethanolic extracts exhibited the best anti-inflammatory activities, with IC50 values of 292.48 ± 13.97 and 420.30 ± 19.80 µg/mL for S. chevalieri and C. pentandra, respectively. Statistical analyses indicated that the observed differences between the species are significant ($P < 0.05$). These values are lower than those reported by Abouelela et al. [20], which indicated that extracts of C. pentandra had anti-inflammatory activity comparable to ascorbic acid. This discrepancy could be due to several factors, including the plant parts used, the extraction solvents employed, and climatic variations in the area where the plants were collected [15, 48, 49]. Further analysis of the extracts of S. chevalieri and C. pentandra should also consider the chemical composition of the extracts. Previous studies have shown that flavonoids and tannins play a significant role in the anti-inflammatory activity of plants [13, 21, 50]. A more detailed characterization of the bioactive compounds present in the extracts could provide additional insights into the underlying mechanisms of the observed activity.

CONCLUSION

This study demonstrated that S. chevalieri and C. pentandra are the most used species for treating cough in Bamako, Mali. Leaves and branches are the most utilized plant parts for treating these conditions. A decoction is the most employed preparation method. Phytochemical investigation revealed that the leaves of S. chevalieri and the bark of C. pentandra are potential sources of secondary metabolites. The dosage results indicated a relatively high content of polyphenols and total flavonoids in the hydroethanolic extracts of S. chevalieri, while the aqueous extract of C. pentandra exhibited a high content of total flavonoids. Although the extracts of S. chevalieri and C. pentandra showed promising anti-inflammatory activity, these various metabolites and their anti-inflammatory effects likely explain the traditional use of these plants by local populations. However, further research is needed to optimize the characterization of active compounds and assess their efficacy in vivo. These initiatives will advance our knowledge of and ability to use medicinal plants to reduce the inflammation linked to children's coughs.

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ETHIC STATEMENT: None

REFERENCES

1. Canning BJ, Chang AB, Bolser DC, Smith JA, Mazzone SB, McGarvey L. Anatomy and neurophysiology of cough: CHEST guideline and expert panel report. Chest. 2014;146(6):1633-48.
2. Andrani F, Aiello M, Bertorelli G, Crisafulli E, Chetta A. Cough, a vital reflex. Mechanisms, determinants, and measurements. Acta Biomed. 2018;89(4):477.
3. Saadat S, Shakeri F, Boskabady MH. Comparative antitussive effects of medicinplants and their constituents. Altern Ther Health Med. 2018;24(4):36-49.
4. Kané B, Camara M, Dembélé G, Togo S, Traoré M, Diallo K, et al. Epidemiological aspect of community acute pneumopathy of children in the pediatric service of Mali Hospital. Rev Malienne d'infectiologie Microbiol. 2020;10(1):64-70.
5. Yadav S, Mishra AP, Kumar S, Negi A, Asha, Maurya VK. Preparation of phytopharmaceuticals for the management of disorders: The development of nutraceuticals and traditional medicine. Elsevier; 2021. pp. 169-84. doi:10.1016/B978-0-12-820284-5.00007-1
6. Ganga NP. The therapeutic applications of cough medicines in respiratory diseases. Int J Pharm Phytopharmacol Res. 2021;11(3):6-10.
7. OMS. Sirop contre la toux et le rhume: l'OMS ouvre une enquête après la mort de 66 enfants en Gambie [Internet]. OMS; 2022 [updated 2022 October 6; cited 2024 september 23].
8. Chaachouay N, Zidane L. Plant-derived natural products: A source for drug discovery and development. Drugs Drug Candidates. 2024;3(1):184-207.

9. Chopra B, Dhingra AK. Natural products: A lead for drug discovery and development. Phytother Res. 2021;35(9):4660-702.
10. Diallo BN, Swart T, Hoppe HC, Bishop ÖT. Potential repurposing of four FDA-approved compounds with antiplasmodial activity identified through proteome scale computational drug discovery and in vitro assay Groningen Machine for chemical simulations. Sci Rep. 2021;11(1413):1-15.
11. Diallo BN, Glenister M, Musyoka TM, Lobb K, Bishop ÖT, Tastan Bishop Ö. SANCDB: An update on South African natural compounds and their readily available analogs. J Cheminform. 2021;13(1):1-14.
12. Simoben CV, Qaseem A, Moumbock AFAA, Telukunta KK, Günther S, Sippl W, et al. Pharmacoinformatic investigation of medicinal plants from East Africa. Mol Inform. 2020;39(11):1-15.
13. Konaré MA, Condurache NN, Togola I, Păcularu-Burada B, Diarra N, Stănciuc N, et al. Valorization of bioactive compounds from two underutilized wild fruits by microencapsulation in order to formulate value added food products. Plants. 2023;12(2):267.
14. Konaré MA, Diadie Maïga A, Togola I, Diarra N. Antioxidant and antidiabetic potential of extracts from Anacardium occidentale and Sclerocarya birrea. J Biochem Technol. 2024;15(3):15-24.
15. Majolo F, de Oliveira Becker Delwing LK, Marmitt DJ, Bustamante-Filho IC, Goettert MI. Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. Phytochem Lett. 2019;31(5):196-207.
16. Ntie-Kang F, Telukunta KK, Döring K, Simoben CV, A. Moumbock AF, Malange YI, et al. NANPDB: A resource for natural products from Northern African sources. J Nat Prod. 2017;80(7):2067-76.
17. Togola I, Dembélé AA, Tounkara F, Diarra N, Konaré MA, Karembé M, et al. Evaluation of in vitro antioxidant activities of ethanol extracts of Datura innoxia Mill. Leaves and Seeds Harvested in Mali. Ann Res Rev Biol. 2019;33(2):1-8.
18. Togola I, Konaré MA, Diakité M, Diarra N, Tounkara F, Sanogo R, et al. Evaluation de la teneur en alcaloïdes totaux a differents stades de developpement de Datura innoxia Mill., une plante utilisee dans la medecine traditionnelle au Mali. Am J Innov Res Appl Sci. 2019;9(2).
19. Pal M, Das R, Ghosh N, Kundu A, Saha A, Sharma C, et al. Effect of some medicinal plants as antitussive potential- A review. Int J Biol Pharm Allied Sci. 2024;13(5):2073-81.
20. Abouelela ME, Orabi MAA, Abdelhamid RA, Abdelkader MS, Madkor HR, Darwish FMM, et al. Ethyl acetate extract of Ceiba pentandra (L.) Gaertn. Reduces methotrexate-induced renal damage in rats via antioxidant, anti-inflammatory, and antiapoptotic actions. J Tradit Complement Med. 2020;10(5):478-86.
21. Orabi MAA, Abouelela ME, Darwish FMM, Abdelkader MSA, Elsadek BEM, Al Awadh AA, et al. Ceiba pentandra ethyl acetate extract improves doxorubicin antitumor outcomes against chemically induced liver cancer in rat model: A study supported by UHPLC-Q-TOF-MS/MS identification of the bioactive phytomolecules. Front Pharmacol. 2024;15:1337910.
22. Islamoglu MS, Uysal BB, Yavuzer S, Cengiz M. Influence of the use of medicinal plants on the level of medication adherence in the Elderly. Int J Pharm Phytopharmacol Res. 2024;14(1):16-22.
23. Guindo F, Konaré MA, Daou C, Cissé F. Inventory of local practices of use and conservation of baobab products in two regions of Mali. J Food Stud. 2022;11(1):21.
24. Chiribagula B, S BA, E NI, E KM, Ndjolo P, J KB, et al. Criblage phytochimique et activité antimicrobienne de sept fleurs comestibles utilisées en médecine traditionnelle à Lubumbashi (RDC). J Appl Biosci. 2018;2(1).
25. Konaré MA, Cissé C, Togola I, Fomba M. Inhibitory effects of Datura innoxia Mill extracts on two enzymes involved in the control of diabetes and inflammation. Annu Res Rev Biol. 2024;39:11-8.
26. Gambhire M, Juvekar A, Wankhede S. Evaluation of anti-inflammatory activity of methanol extract of Barleria Cristata leaves by in vivo and in vitro methods. Internet J Pharmacol. 2008;7(1):1-4.

27. Koné MO, Togola I, Konaré MA, Diarra N, Sanogo R, Karembé M. In vitro, anti-inflammatory, and anti cholesterolemic activities of Chrysanthellum americanum (L) vatke harvested in mali. Int J Biochem Res Rev. 2022;31(10):91-9.
28. Begbin EK, Odoh EA, Zougrou EG, Kablan CLA, Kouakou KK. Évaluation des activités hypoglycémiantes et anti-hyperglycémiantes de l'extrait aqueux des feuilles de Cnestis ferruginea vahl ex dc. (Connaraceae) chez la souris saine. Rev Ivoir Sci Technol. 2021;37(2021):186-95.
29. Lebri M, Bahi C, Fofie N, Gnahoue G, Lagou S, Achibat H, et al. Analyse phytochimique et évaluation de la toxicité aiguë par voie orale chez des rats de l'extrait total aqueux des feuilles de Abrus precatorius Linn (Fabaceae). Int J Biol Chem Sci. 2015;9(3):1470-6.
30. Chatterjee A, Singh N, Chanu WK, Singh CB, Nagaraj VA. Phytochemical screening, cytotoxicity assessment, and evaluation of in vitro antiplasmodial and in vivo antimalarial activities of Mentha spicata L. methanolic leaf extract. J Ethnopharmacol. 2022;298:115636. doi:10.1016/j.jep.2022.115636
31. Samake S, Togola I, Diarra N, Samake M, Sanogo R. Etude ethnobotanique et screening phytochimique de plantes ornementales à usage therapeutique du district de bamako, mali. Int J Adv Res. 2020;8(8):251-62.
32. Romuald KS, Blaise KW, Konan YA, Djakalia OU. Plantes utilisées dans le traitement traditionnel de l'infertilité féminine dans le département de Dabakala (Côte d'Ivoire). J Anim Plant Sci. 2019;42(1):708699.
33. Elumalai A, Mathangi N, Didala A, Kasarla R, Venkatesh Y. A review on Ceiba pentandra and its medicinal features. Asian J Pharm Technol. 2012;2(3):83-6.
34. Chan EWC, Yeong SW, Wong CW, Soo OYM, Phua ACY, Ng YK. Ceiba pentandra (L.) Gaertn: An overview of its botany, uses, reproductive biology, pharmacological properties, and industrial potentials. J Appl Biol Biotechnol. 2023;11(1):1-7.
35. Silué GNA, Belemlilga MB, Djadji ATL, Traoré KT, Boly GAL, Koala M, et al. Anti-inflammatory and antioxidant activities assessment of an aqueous extract of Ceiba pentandra (L.) Gaertn (Malvaceae). RPS Pharm Pharmacol Rep. 2023;2(4):1-8.
36. Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L. J Food Sci Technol. 2013;50(4):687-95.
37. Ojurongbe O, Nguetse CN, Fayemiwo SA, Falade CO, Ojurongbe TA, Thomas BN, et al. High prevalence of dihydrofolate reductase gene mutations in Plasmodium falciparum parasites among pregnant women in Nigeria after reported use of sulfadoxine-pyrimethamine. Pathog Glob Health. 2018;112(2):86-92.
38. Tala VRS, Tcheghebe OT, Happi MZY, Ernest T, Munvera AM, Mkounga P, et al. Phytochemical screening and antibacterial potential of the trunk bark of Ochthocosmus africanus. Int J Pharm Phytopharmacol Res. 2023;13(6):1-7.
39. Konaré MA, Keita S, Somda MK, Togola I, Diarra N, Sanogo R. Antibacterial activities of extracts from four wild food fruits. Eur J Nutr Food Saf. 2023;15(1):62-71.
40. Keita S, Wélé M, Cisse C, Diarra N, Kirkman L, Baba-Moussa L. Antibacterial and antiplasmodial activities of tannins extracted from Zizyphus mauritiana in mali. Int J Biochem Res Rev. 2018;24(2):1-8.
41. Süntar I. Importance of ethnopharmacological studies in drug discovery: Role of medicinal plants. Phytochem Rev. 2019;19(5):1199-209.
42. Dubey K, Dubey R, Gupta RA, Gupta AK. Anti-diabetic and antioxidant potential of saponin extract of leaves of Zizyphus mauritiana. J Drug Deliv Ther. 2019;9(2-A):75-7.

43. Dembele F, Samake S, Togola I, Karembe M. Etudes ethnobotanique et phytochimique despeces ornementales utilisees au Mali: Cas de Terminalia catappa (L.) dans le district de Bamako. Int J Appl Res. 2021;7(6):01-7.
44. Togola I, Abdoulaye M, Cisse C, Diarra N. Anti-inflammatory and Anti-cancer activities of leaf extracts from three Datura species found in mali. J Appl Life Sci Int. 2023;26(6):88-97.
45. John KK, Shcherazade OSF, Georges A, Ernest ZN, Roger KK, Emile BK, et al. Activité Anti-inflammatoire et études phytochimiques de l'extrait aqueux des écorces de Distemonanthus benthamianus Baill. (Caesalpiniaceae: Leguminosae - Caesalpinioideae). Eur Sci J ESJ. 2021;17(7):74-94.
46. Adjdir S, Benariba N, Laoufi H, Djaziri R. Phenolic content and antioxidant activity of Ziziphus jujuba Mill. Fruit extracts. Phytotherapie. 2019;17(2):74-82.
47. Taiwo BJ, Miller AH, Fielding AJ, Sarker SD, van Heerden FR, Fatokun AA. Ceibinin, a new positional isomer of mangiferin from the inflorescence of Ceiba pentandra (Bombacaceae), elicits a similar antioxidant effect but no anti-inflammatory potential compared to mangiferin. Heliyon. 2024;10(1).
48. Gebrehiwot S, Shumbahri M, Eyado A, Yohannes T. Phytochemical screening and in vivo antimalarial activity of two traditionally used medicinal plants of Afar region, Ethiopia, against Plasmodium berghei in Swiss Albino mice. J Parasitol Res. 2019;2019(1):4519298.
49. Togola I, Denou A, Dembélé J, Daou C, Dénou A, Diarra N, et al. Ethnobotanical survey and phytochemical screening of some plants used in the management of erectile dysfunction in Bwatun (Mali). J Nat Prod Plant Resour. 2020;9(01):1-8.
50. Sorokina M, Steinbeck C. Review on natural products databases: Where to find data in 2020. J Cheminform. 2020;12(1):1-51.

Beyond the Barrier: The Endothelium's Unsung Role in Physiology & Pathology

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ABSTRACT

Research conducted in recent years has significantly transformed our understanding of the role of the vascular endothelium in maintaining the overall homeostasis of the body. It has been revealed that the endothelium is responsible for synthesizing a wide range of biologically active substances that play a key role in numerous processes both in health and disease - such as hemodynamics, hemostasis, immunological responses, and regenerative processes. This extensive endocrine activity has led to the endothelium being sometimes referred to as an "endocrine tree." The functioning of the endothelium depends on its functional condition, which is shaped by the signals it receives. Endothelial dysfunction understood as an impairment of the vasodilatory, antithrombotic, and anti-inflammatory properties of the cells lining the vessels, is closely linked to cardiovascular diseases - the leading cause of death worldwide. It is considered a key stage in the development of atherosclerosis and one of the major risk factors for hypertension, diabetes mellitus, and cardiovascular incidents. This paper aims to gather and present information that will allow for a better understanding of the structure and significance of this majestic organ in human physiology.

Keywords: Endothelium, Endothelial dysfunction, Cardiovascular system, Oxidative stress

INTRODUCTION

Over the past three decades, our understanding of the role of the vascular endothelium has continuously changed, recognizing it as a complex organ that is dynamically controlled and essential for a variety of physiological and pathological processes. Initially perceived as a simple semipermeable barrier—"the cellophane wrapper" of the vascular tree—recent abundant research underscores its critical physiological functions, particularly in regulating vascular tone, blood flow, and platelet activation [1, 2]. Simple squamous cells with a cobblestone-like appearance, endothelial cells (ECs) line the whole cardiovascular system (CVS), including the blood vessels, lymphatic vessels, and walls of the heart, in a continuous, thin, and smooth monolayer [1, 3, 4]. The endothelium is home to numerous receptors for various biologically active substances (BAS), and it also senses the pressure and volume of the moving blood - a phenomenon known as shear stress, which stimulates the synthesis of anticoagulant and vasodilatory substances. Therefore, the higher the pressure and speed of the blood flow (in arteries), the less likely clots are to form. Endothelial dysfunction (ED), which occurs under the influence of damaging factors (mechanical, infectious, metabolic, immune complex, etc.), rapidly reverses the direction of its hormonal activity: vasoconstrictors and coagulants are produced [1-4].The biologically active substances produced by the endothelium mainly act in a paracrine manner (on neighboring cells) and autocrine-paracrine manner (on the endothelium itself), but the vascular wall is a dynamic structure. Its endothelium is continuously renewed, and as parts become obsolete, they, along with BAS, enter the bloodstream, spread throughout the body, and can affect systemic blood flow. The activity of the endothelium can be assessed based on the concentration of BAS in the blood. The structure of the vascular wall creates a certain pattern in the distribution of coagulating factors (vasoconstrictors) and anticoagulant factors (vasodilators) [2]. As long as the endothelium remains intact and undamaged, it

primarily synthesizes anticoagulant factors, which also serve as vasodilators. They provide a non-thrombogenic surface with highly selective permeability properties, actively regulating molecule exchange in response to endogenous or exogenous signals. Covering an approximate surface area of 4000-7000 m2, the endothelium is one of the body's largest organs, highlighting the significant impact its dysfunction can have on body homeostasis [2]. Pathologically, the endothelium serves dual roles: it mediates immune responses by amplifying inflammation at injury or infection sites, and as an integral component of the cardiovascular system, its dysfunction can lead to disease [2, 3]. This paper aims to gather and present information that will allow for a better understanding of the structure and significance of this majestic organ in human physiology.

RESULTS AND DISCUSSION

Endothelial dysfunction

There are two types of blood vessels: macrovasculature and microvasculature. Large blood vessels, including arteries and veins, that carry blood to and from organs make up the macrovasculature. The ECs that make up the majority of the microvasculature are tiny arteries, venules, and capillaries. It is crucial for managing the metabolic exchanges between the blood and peripheral tissues as well as local blood perfusion. Arterioles, measuring 10100 μm in diameter, are highly innervated and respond to sympathetic vasoconstriction, crucial for adjusting vascular peripheral resistance and blood flow volume, thereby influencing capillary fluid exchange. Capillaries and venules are the primary sites for fluid and macromolecular exchanges, with venules also playing a significant role in leukocyte adhesion. This indicates that ED in various locations can disrupt numerous physiological processes within the organism [5].

ED is identified as a pathological state marked by an imbalance between vasodilating and vasoconstricting substances, a loss of anti-thrombotic properties, and increased permeability. It encompasses any form of abnormal endothelial activity. Most cardiovascular (CV) risk factors are associated with ED, and their therapeutic modification can lead to improvements in vascular function [3, 4]. A common manifestation of ED is impaired nitric oxide (NO) bioavailability, resulting from either reduced production by endothelial nitric oxide synthase (eNOS) or increased degradation by reactive oxygen species (ROS) [4].

A summary of the interactions and mechanisms of action of various endothelium-derived factors on endothelial cells is presented in **Figure 1.**

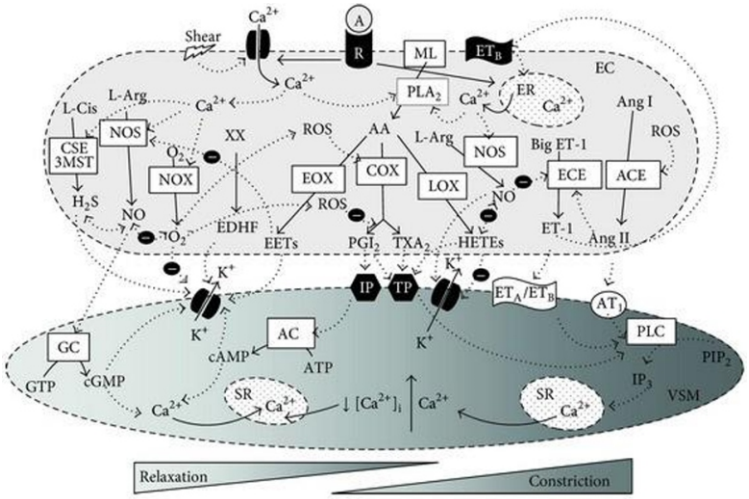


Figure 1. A summary of the interactions and mechanisms of action of various endothelium-derived factors on endothelial cells. The image adopted from Bernatova [6]

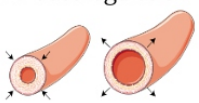
ED is a hallmark of cardiovascular disease and arteriosclerotic vascular disease, commonly known as atherosclerosis, where it has been consistently documented. The dysregulation of endothelial cells plays a pivotal role in a multitude of pathological conditions including vasculitis, hypertension, cardiomyopathy, retinopathy, neuropathy, and cancer. ED is not limited to hypertensive individuals but is also observed in normotensive subjects with a family history of hypertension, as well as among active and passive smokers, and conditions like aging, obesity, diabetes mellitus, dyslipidemia, hyperhomocysteinemia, and in patients with inflammatory or infectious diseases. Often, these conditions are linked to an overproduction of ROS leading to oxidative stress, which interacts with nitric oxide reducing its availability and potentially causing direct cellular damage through the production of peroxynitrite. Thus, oxidative stress is a critical mechanism in the development of ED, if not its primary cause [5-7].

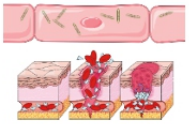
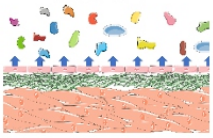
The significance of endothelium in physiology and pathology – molecular mechanisms

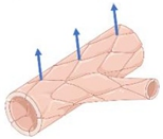
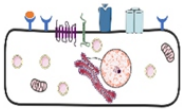
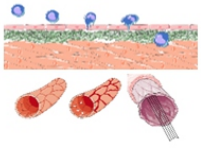
Besides the abovementioned facts, the endothelium plays a crucial role in the innate immune system, acting as a barrier to prevent the intrusion and systemic spread of pathogens Tight joints, which quickly mend after a vessel burst, preserve the integrity of this barrier. Additionally, the endothelium exerts a significant paracrine function, secreting chemokines, interleukins, interferons, and growth factors, and it facilitates the recruitment and extravasation of immune cells at inflammation sites. Critical to this process are adhesion molecules such as Eselectin, P-selectin, intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM), present on ECs surface. It has been observed that the four classical signs of inflammation - calor (heat), rubor (redness), tumor (swelling), and dolor (pain) - are regulated by EC responses to stimuli [5, 6, 8]. Furthermore, the endothelium forms the lymphatic vessels, essential components of the peripheral immune system. These blindended tubules, equipped with valves, facilitate the drainage of interstitial fluid containing CD45+ cells and potential pathogens. Although ECs are integral to the immune system, they do not possess the classical immune cell functions such as killing, phagocytosis, or antibody production. Nonetheless, they are an essential part of the body's defense system, and their dysfunction can lead to dysregulation of immune processes, a common occurrence in numerous diseases.

Table 1 depicts the fundamental functions of endothelium that are key in the maintenance of the body's homeostasis. All those processes could be impaired in ED, which could lead to serious issues that impact the entire body.

Table 1. The main functions played by endothelial cells (ECs) are illustrated by Servier Medical Art.

1. Vasoregulation 	By preserving the balance of vasodilative and vasoconstrictive impulses, the endothelium contributes significantly to the regulation of vascular tone, allowing BP and blood flow to adjust to the demands of the moment. By delivering paracrine signals to the smooth muscle cells encircling the arteries, ECs regulate their vasoregulation by either contracting or relaxing them. The most potent vasoconstrictor is endothelin, a 21aa peptide existing in three isoforms – mainly produced by ECs, whereas its main counterplayer is a gasotransmitter – NO. Under normal conditions, eNOS provides NO to adjust vascular tone to alter BP and blood flow. In ED, NO bioavailability is impaired which leads to the development of atherosclerosis and CVD.

2. First aid kit - role in hemostasis 	During the coagulation process, ECs play a crucial role in providing the proper hemostatic balance. Besides the principal role of providing a non-thrombogenic inner layer of the vascular wall, ECs also provide some of the substances essential to the process. Weibel-Palade bodies (WPB) are rod-shaped subcellular organelles considered endothelial-specific first aid kits, equipped to provide an efficient response to the damage sustained by the vasculature, without losing time on the translation process. Their major constituent is vWF whose base role is to recruit the platelets in the place of injury. The remaining content is P-selectin which recruits leukocytes to guard the wound, IL-8 to boost inflammation and recruit neutrophils, ET for vasoconstriction in the affected area, angiopoietin-2 which helps in tissue repair and tPA which prevents excessive fibrin formation. WPB exocytosis is triggered by fIIa, VEGF, or epinephrine. Intense exocytosis of WPB likely promotes vascular inflammation and atherosclerosis. WPB is thought to be the most active promoter of platelet and leukocyte adhesion.
3. Angiogenesis 	Angiogenesis is required during growth and development as well as in tissue repair and restoration of oxygen and metabolite supply. During angiogenesis, activated ECs undergo mitosis and migrate out from a preexisting vessel towards a gradient of VEGF. It is a growth factor that is produced autocrinally by ECs or paracrinally by inflammatory cells under hypoxic conditions and stimulates mentioned processes in the endothelium. Other factors act angiogenically such as the large family of angiopoietin peptides or chemokines, e.g., IL-8 that induces the proliferation of ECs in ischemic myocardium or different cancers. New vessels' formation begins as outgrowing sprouts of tip and stalk cells. Tip cells equipped with many filopodia and VEGF receptors to sense the growth factor gradient are highly migratory leading cells behind which follow the stalk cells. Among three receptors for VEGF-A: VEGFR1, VEGFR2, and VEGFR3, belonging to the tyrosine kinase family, VEGFR2 seems to play first fiddle in this process even though VEGFR1 has greater affinity for VEGF. VEGF-C and VEGF-D interacting with VEGFR3 on the surface of lymphatic ECs leads to lymphangiogenesis.
4. Secretion 	ECs secrete chemokines, interleukins, interferons, growth factors, and vasoactive substances, all of which can play a role in metabolic homeostasis. Galley and Webster grouped the factors depending on their function: vasodilating factors – NO and prostacyclin, and the vasoconstricting compounds such as TXA2, free radicals, leukotrienes, or ET. Other emphasized substances mentioned in this study are factors acting on the hemostasis process: procoagulants e.g., vWF, TXA2, fV, thromboplastin, PAF or PAI-1 and antithrombotics including prostaglandins, thrombomodulin, antithrombin, as well as uPa. In turn, ECs are also involved in the production of the substances that constitute the extracellular matrix, mostly fibronectin, laminin, collagen, and proteoglycans. Moreover, endothelium produces growth factors like IGF; HGF; CTGF/CCN2; TGF-β; colony-stimulating factors, or VEGF. Lastly, it is worth mentioning that PRR stimulation leads to the production of inflammatory mediators including IL-1β, IL-6 and IL-8, TNF-α, and leukotrienes. The WBP has a vital role in EC secretion, but its content remains situation-dependent.

5. Glucose and lipid transport. 	By modifying transendothelial access and local blood flow, glucose transport is controlled. The primary GLUT in ECs, the GLUT-1 transporter, plays a crucial role in the energy-independent transport of glucose across the cellular membrane. It is noteworthy that certain mediators, such as the HIF-1α factor, cause an increase in GLUT-1 expression. However, insulin signaling does not control glucose transport in human micro- and macrovascular ECs. Another important organ in the control of lipid transport in the body is the endothelium. It is not necessary for fatty acids (FA) with medium (6–12 carbons) and short (< 6 carbons) chains to actively traverse the cell membrane. Compared to long-chain FA (> 12 carbons), which require a specific transporter to get across the cell membrane, they are less common in diets. Before the mAspAT/FABPPM transporter was identified in rat aortic ECs in 1991, it was believed that all lipids could enter the cells by diffusion. Since then, more types of FA transport proteins (FATPs) have been identified, such as intracellular FABPs (FA-binding proteins), FATP1-6, and FAT/CD36 (FA translocase). When VEGF-B, for example, is produced by nearby cardiomyocytes, it causes the expression of FATP3 and FATP4, which helps ECs absorb FA, which is necessary for the synthesis of ATP. Very relevant is the fact that lipoproteins are transported across endothelium via coordinated receptor-mediated endocytosis and exocytosis. All LDL receptors, scavenger receptors, and VLDL receptors can take up the LDL, acetylated LDL, oxidized LDL (oxLDL), or VLDL. CD36 is a key scavenger receptor that is required for the internalization of oxLDL in ECs. In turn, HDL transport is possible thanks to SR-B1 and ABCG1 proteins.
6. Receptor and marker organ 	As endothelium is one of the biggest body organs, it is equipped with an abundance of surface proteins for various purposes. ECs express several PAMP receptors including the TLR family. TLR1-TLR6 and TLR9 are found in all different kinds of tissue-specific ECs in rest, whereas TLR7, TLR8, and TLR10 are normally absent but could be induced under inflammatory activation. Their activation stimulates the production of cytokines by endothelium. Another characteristic feature of ECs is the presence of membrane-bounded receptors for numerous ligands including proteins, lipid-transporting particles, metabolites, and hormones. The junctional proteins that provide cell-cell and cell-matrix interactions are also widely expressed among ECs. In inflammation, the expression of adhesion molecules is increased and more E-selectin, P-selectin, ICAM and VCAM molecules provide easier leukocyte migration. ECs also form tight junctions between themselves thanks to several proteins including claudin-5, and its reduction in junctions leads to elevation of vascular permeability. ECs also express on their membranes the blood group AB antigens which should always be considered in transplantations.
7. Leukopheresis and cell migration barrier 	Leukapheresis is the first step of the multistage process of extravasation of leukocytes to the site of inflammation or infection, and its regulation is one of the most crucial functions of ECs during inflammatory response. For ECs, it is a major challenge to maintain the balance between tightly sealing the vessel walls to prevent leakage of transported fluid on the one hand and facilitating extravasation of immune cells on the other. Transendothelial migration (TEM) takes place in several stages (1) tethering, (2) rolling (3) firm adhesion, (4) crawling, and eventually (5) diapedesis. The main group of adhesion molecules is constituted by Ca^{2+} dependent lectins: E-selectin that must be synthesized de novo after IL-1 or TNFα induction, P- selectins that are stored in WPBs, whereas L-selectin is synthesized constitutively by leukocytes. The key role is also played by chemokines such as CCL2 that elicits a rapid surface presence of L-selectin, PSGL-1, and CD44 crucial for the neutrophil recruitment process and induces E-selectin expression to attach to monocytes. Arrest and crawling are mediated by stronger anchoring through integrin and their ligands: ICAM-1 and LFA-1; VCAM-1 and VLA-4 which provide the cell-cell and cell-ECM interaction supporting the crawling process even against the flow direction. The passage of leukocytes is paracellular in 90% of cases, however, 10% of TEM is happening transcellular directly through ECs. It additionally implies why the non-disturbed function of ECs is essential in leukocyte migration.

Abbreviations used in **Table 1,** not explained in the text: CCL2 – chemokine C-C motif ligand 2, CTGF/CCN2 – connective tissue growth factor, ET – endothelin, fIIa – active factor II – thrombin, FATP – fatty acid transport proteins, fV – factor V, GLUT-1 – glucose transporter 1, HGF – hepatocyte growth factor, HIF-1α – hypoxia-inducible factor 1-alpha, ICAM – intercellular adhesion molecule 1, IL – interleukin, IGF – insulin-like growth factor, LFA-1 – lymphocyte function-associated antigen 1, LDL – low-density lipoproteins, mAspAT/FABPPM – mitochondrial aspartate aminotransferase/fatty acid binding protein (pm – plasma membrane), oxLDL – oxidized LDL, PAF – plateletactivating factor, PAMPs – pathogen-associated molecular patterns, PRR – pattern recognition receptors, PSGL-1 – P-selectin glycoprotein ligand-1, SR-B1 – scavenger receptor class B, type 1, TGF-β – transforming growth factor β, TLR – toll-like receptor, TNFα – tumor necrosis factor α, tPA – tissue plasminogen activator, TXA2 – thromboxane A2, uPA – urokinase, VLA-4 – very late antigen-4 (integrin α4β1), VEGFR1/2/3 – VEGF receptor 1/2/3, VLDL – very low-density lipoproteins.

Increased oxidative stress, primarily due to an overproduction of ROS, is recognized as a key mechanism in the development of ED. The aberrant synthesis of vasoactive molecules caused by this oxidative stress further raises the risk of CV disease and impairs vasoregulation [9]. Vessel constriction results from an imbalance brought on by decreased secretion of vasodilatory factors and a predominance of vasoconstrictive ones. If this imbalance persists for a long time, total peripheral resistance may rise, raising blood pressure (BP) throughout the body [10]. This assertion is supported by studies in animal models that examined the consequences of eNOS gene deletion and chronic inhibition of NO synthesis with Nω-nitro-L-arginine methyl ester, both of which were found to induce arterial hypertension [11-13]. However, the relationship between ED and hypertension is complex, potentially forming a vicious cycle where high BP exacerbates ED through increased shear stress on endothelial cells, thus highlighting the importance of antihypertensive therapy to mitigate this process [11].

Virchow's triad is a renowned concept in medicine regarding the formation of thrombosis. It is a combination of three factors that underlie the development of blood clots within the blood vessels. Those three primary factors are endothelial dysfunction or damage, altered blood flow, and intrinsic hypercoagulability. In ED, the balance between prothrombotic and antithrombotic factors is often disrupted, necessitating pharmacological intervention to restore homeostasis [14, 15]. Initiated by the unpacking of a vascular emergency kit, the release of WeibelPalade bodies (WPB) occurs. WPB are secretory organelles found in endothelial cells lining the intima of arteries, capillaries, veins, and the endocardium. WPB store factors regulating vascular hemostasis—the release of WPB leads to an increase in local serum levels of von Willebrand factor (vWF) and elevated presence of P-selectin on the surface of activated ECs [15, 16]. An increase in vWF levels is implicated in thrombotic events, whereas Pselectin facilitates the initial recruitment of leukocytes to sites of injury or inflammation [17, 18]. P-selectin also plays a significant role in the development of arteriosclerotic vascular disease [17, 19], though the direct link between WPB exocytosis and atherosclerotic plaque formation requires further elucidation.

Angiogenesis disruption is another consequence of ED. Age-related ED may cause endothelial apoptosis in the microvasculature, impair the function of endothelial progenitor cells (EPCs), and downregulate vascular endothelial growth factor (VEGF), a crucial regulator of angiogenesis across various tissues [20]. Cardiovasculardiseases (CVDs), such as coronary artery disease, can diminish the function and number of circulating EPCs. Moreover, disruptions in angiogenesis within the context of CVD pose significant risks, as they may hinder the formation of compensatory circulation, thereby

limiting blood supply to cardiac muscle cells. The impact of angiogenesis disturbances is also pronounced in cancer, where hypoxic or damaged cancer cells release VEGF in a paracrine manner. It is believed that the majority of EPCs originate from the CD133+ hemangioblast stem cellpopulation, mobilized into circulation in response to VEGF or granulocyte-macrophage colony-stimulating factor [21]. ED serves as a foundational step in the progression of arteriosclerotic vascular disease, playing a pivotal role in its development. Conditions such as hypertriglyceridemia, dyslipidemia, and diabetes mellitus are wellestablished contributors to ED. In the context of ED, the increased surface expression of adhesive molecules due to inflammation and oxidative stress amplifies the influx of immune cells, exacerbating atherogenesis [21]. This process is characterized by chronic dysregulation of adhesion molecules, allowing immune cells to bypass the cell migration barrier. Additionally, receptor dysregulation and secretion imbalances are implicated in the onset of metabolic disorders, including obesity, insulin resistance, dyslipidemia, cognitive impairment, diabetes, and fatty liver disease [7]. The consequences of ED are illustrated in **Figure 2.** In inflammatory conditions, ED arises from a complex interplay among the endothelium, pro-inflammatory cytokines, circulating lipids, platelets, and traditional CV risk factors. Endothelial cells can be activated by these substances directly or indirectly, which compromises their functionality and promotes a pro-atherogenic state. Subclinical vascular damage and the development of clinically evident cardiovascular disease are the final results of this state.

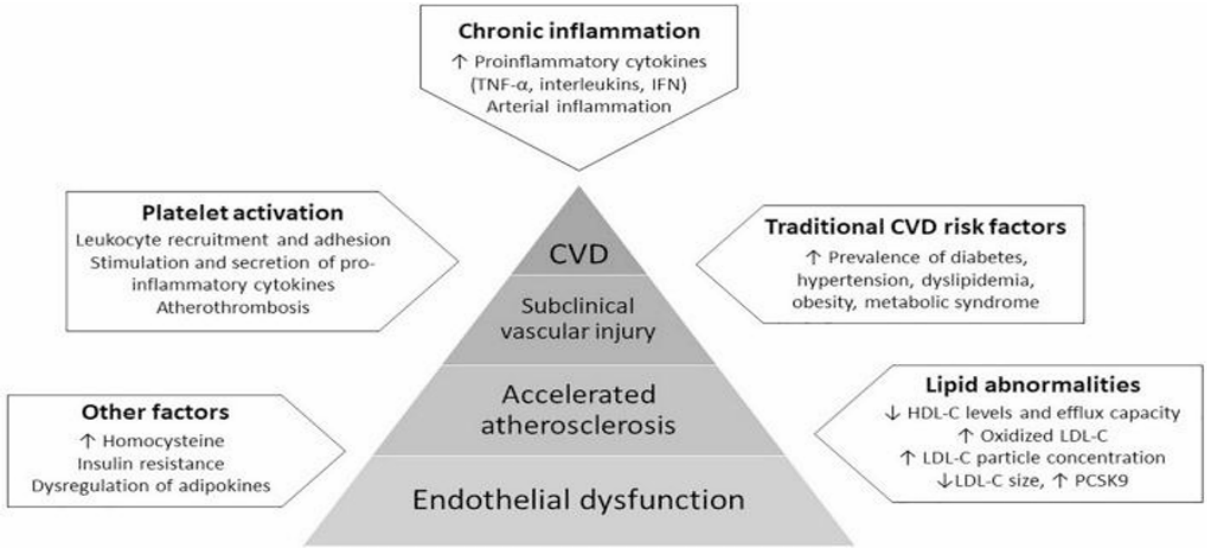


Figure 2. Outline of the clinical and pathophysiological repercussions of endothelial dysfunction. The image is sourced from Anyfanti et al. [22]

CONCLUSION

The relationships highlighted indicate that in ED, the processes described above are compromised to varying extents. Atherosclerosis, hypertension, dyslipidemia, and altered coagulation system characteristics are all significantly linked with CVD. Consequently, addressing ED through pharmacological therapy indirectly targets these conditions, enhancing the quality of life and survival rates of affected individuals. ED not only represents a CV risk in itself but also maintains strong associations with other CV conditions. The endothelium is thought to be one of the most significant organs in the human body, and almost any illness may cause it to malfunction. It is impossible to

overestimate the importance of this organ in preserving physiological homeostasis, and researchers, health professionals, and non-health professionals alike ought to pay it greater attention.

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REFERENCES

1. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, et al. The vascular endothelium and human diseases. Int J Biol Sci. 2013;9(10):1057-69.
2. Flammer AJ, Lüscher TF. Three decades of endothelium research: From the detection of nitric oxide to the everyday implementation of endothelial function measurements in cardiovascular diseases. Swiss Med Wkly. 2010;140(4748):w13122.
3. Sturtzel C. Endothelial cells. Adv Exp Med Biol. 2017;1003:71-91.
4. Brutsaert DL, Andries LJ. The endocardial endothelium. Am J Physiol. 1992;263(4 Pt 2):H985-1002.
5. Pi X, Xie L, Patterson C. Emerging roles of vascular endothelium in metabolic homeostasis. Circ Res. 2018;123(4):477-94.
6. Bernatova I. Endothelial dysfunction in experimental models of arterial hypertension: Cause or consequence? Biomed Res Int. 2014;2014(1):598271.
7. Galley HF, Webster NR. Physiology of the endothelium. Br J Anaesth. 2004;93(1):105-13.
8. Konukoglu D, Uzun H. Endothelial dysfunction and hypertension. Adv Exp Med Biol. 2017;956:511-40.
9. Figueiredo VN, Yugar-Toledo JC, Martins LC, Martins LB, de Faria AP, de Haro Moraes C, et al. Vascular stiffness and endothelial dysfunction: Correlations at different levels of blood pressure. Blood Press. 2012;21(1):31-8.
10. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995;377(6546):239-42.
11. Arnal JF, El Amrani AI, Chatellier G, Ménard J, Michel JB. Cardiac weight in hypertension induced by nitric oxide synthase blockade. Hypertension. 1993;22(3):380-7.
12. Hermann M, Flammer A, Lüscher TF. Nitric oxide in hypertension. J Clin Hypertens. 2006;8(12 Suppl 4):17-29.
13. Monie DD, DeLoughery EP. Pathogenesis of thrombosis: Cellular and pharmacogenetic contributions. Cardiovasc Diagn Ther. 2017;7(Suppl 3):S291-8.
14. Nightingale TD, McCormack JJ, Grimes W, Robinson C, Lopes da Silva M, White IJ, et al. Tuning the endothelial response: Differential release of exocytic cargos from Weibel-Palade bodies. J Thromb Haemost. 2018;16(9):1873-86.
15. Schillemans M, Karampini E, Kat M, Bierings R. Exocytosis of Weibel-palate bodies: How to unpack a vascular emergency kit. J Thromb Haemost. 2019;17(1):6-18.
16. Dole VS, Bergmeier W, Mitchell HA, Eichenberger SC, Wagner DD. Activated platelets induce WeibelPalade-body secretion and leukocyte rolling in vivo: Role of P-selectin. Blood. 2005;106(7):2334-9.

17. Lowenstein CJ, Morrell CN, Yamakuchi M. Regulation of Weibel-palate body exocytosis. Trends Cardiovasc Med. 2005;15(8):302-8.
18. Ye Z, Zhong L, Zhu S, Wang Y, Zheng J, Wang S, et al. The P-selectin and PSGL-1 axis accelerates atherosclerosis via activation of dendritic cells by the TLR4 signaling pathway. Cell Death Dis. 2019;10(7):507.
19. Ungvari Z, Tarantini S, Kiss T, Wren JD, Giles CB, Griffin CT, et al. Endothelial dysfunction and angiogenesis impairment in the aging vasculature. Nat Rev Cardiol. 2018;15(9):555-65.
20. Wang HW, Lo HH, Chiu YL, Chang SJ, Huang PH, Liao KH, et al. Dysregulated miR-361-5p/VEGF axis
in the plasma and endothelial progenitor cells of patients with coronary artery disease. PLoS One. 2014;9(5):e98070.
21. Kajikawa M, Higashi Y. Triglycerides and endothelial function: Molecular biology to clinical perspective. Curr Opin Lipidol. 2019;30(5):364-9.
22. Anyfanti P, Margouta A, Goulas K, Gavriilaki M, Lazaridou E, Patsatsi A, et al. Endothelial dysfunction in psoriasis: An updated review. Front Med. 2022;9:864185.

Design and Synthesis of Functionalized 2,4-Diamino-1,3,5-Triazines, Potential Inhibitors Involved in Immune and Inflammatory Response

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ABSTRACT

The present study aimed to design and synthesize functionalized 2,4-diamino-1,3,5-triazines, potential inhibitors involved in immune and inflammatory responses. A two-step synthesis method, initially using a microwave reactor for the preparation of reaction intermediates, allowed us to synthesize eleven 6-aryl-2,4-diamino-1,3,5-triazines 5a-k. The intermediates were biguanide derivatives 3a-f which were synthesized under microwave irradiation for 10 min at 130 °C. The 2,4-diamino-1,3,5-triazine derivatives (5a-k) were obtained with yields from 16-86%. The compounds synthesized were submitted to biological evaluation on twelve protein kinases, through the implementation of a dose-response method which allowed the determination of median inhibitory concentration or IC_{50}. Indeed, enzymatic activities were carried out in the presence of 10 µM of ATP, in a final volume of 6 µl for 30 min at 30 °C in ADP-Glo buffer. Among the triazines synthesized, results of enzymatic activity showed that the most active molecule was compound 5b which inhibited PIM1 kinase with IC50 = 1.18 µg/mL. This result is the starting point of a larger research program for our group which could investigate the introduction of the substituted group on triazine's terminal amino group.

Keywords: Protein kinases, Inhibitors, Immunity, Inflammation, Triazines

INTRODUCTION

Protein kinases (PK) have been shown to play a central role in cell survival and regulatory processes, mainly at the level of proliferation mechanisms and immune response [1-5]. As a result, they constituted a set of increasingly sought-after targets in the treatment of cancers, and inflammatory and autoimmune diseases [6-12]. Thus, as part of the development of new so-called targeted therapies, we carried out the design and synthesis, in two steps, of new compounds with 2,4-diamino-1,3,5-triazine moiety. The literature described several routes to access these compounds [13-15] from arylamine or aryl nitrile derivatives with some ester derivates using a microwave reactor [16, 17]. The present study aimed to design and synthesize functionalized 2,4-diamino-1,3,5-triazines, potential inhibitors involved in immune and inflammatory responses. We reported here the results of syntheses and biological

evaluation of these compounds on several protein kinases.

MATERIALS AND METHODS

All commercial reagents were used for our syntheses without any further purification. All solvents were reagent or HPLC grade. Analytical TLC was performed on silica gel 60 F_{254} plates. Column chromatography was carried out on silica gel Merck 60 (70-230 mesh ASTM) and flash Chromatography Grace Reveleris X2™. Melting points were determined on the electrothermal IA 9000 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using a Bruker AVANCE 400 MHz spectrometer. Chemical shift values were reported in parts per million (ppm) and coupling constants (J) were given in hertz (Hz). Multiplicities were reported as follows: br s = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. LC-MS analyses were run on Waters ACQUITY UPLC-MS system composed of a single quadrupole detector (SQD), mass spectrometer (MS) equipped with an electrospray ionization interface (ESI), and a photodiode array detector (PDA).

General procedure for the synthesis of biguanides

To a solution of dicyandiamide (252 mg, 3 mmol, 1 eq) in 5 mL of acetonitrile, was added the corresponding amine derivative (1 eq). Trimethylsilyl chloride or TMSCl (1.1 eq) was added dropwise to the reaction mixture. Stirring was maintained for a few min to obtain the most homogeneous mixture. This mixture, contained in a borosilicate glass vial of 35 ml equipped with snap caps, was put in a CEM Discover SP single-mode microwave reactor for 10 min at 130 °C, under pressure of 18 bar and power of 60 Watt. After 10 min, the reaction mixture was cooled in ice crushed and the precipitate formed was filtered, washed with acetonitrile or isopropanol, and dried. The biguanide derivatives (3a-f) were obtained as powders and were used with further purification for the next step of synthesis.

4-methylthio-phenyl-biguanide (3a)

Compound 3a was obtained according to the general procedure, as a purple powder from dicyandiamide 2 (252 mg, 3 mmol), 4-(methylthio) aniline 1a (0.373 mL, 3 mmol), and TMSCl (0.417 mL, 3.3 mmol).

4-Ethymorpholinyl-biguanide (3b)

Compound 3b was obtained according to the general procedure, as a coral powder from dicyandiamide 2 (504 mg, 6 mmol), N-ethyl-amino-morpholine 1b (0.8 mL, 6 mmol), and TMSCl (0.834 mL, 6.6 mmol).

4-Cyano-phenyl-biguanide (3c)

Compound 3c was obtained according to the general procedure, as an orange powder from dicyandiamide 2 (504 mg, 6 mmol), 4-aminobenzonitrile 1c (708.84 mg, 6 mmol), and TMSCl (0.834 mL, 6.6 mmol).

Benzo[d][1,3]dioxol-5-biguanide (3d)

Compound 3d was obtained according to the general procedure, as a purple powder from dicyandiamide 2 (613 mg, 7.29 mmol), 3,4-(methylenedioxy)aniline 1d (1g, 7.29 mmol), and TMSCl (1.01 mL, 8.02 mmol).

2,4-Dichloro-phenyl-biguanide (3e)

Compound 3e was obtained according to the general procedure, as a white powder from dicyandiamide 2 (311.38 mg, 3.70 mmol), 2,4-dichloroaniline 1e (600 mg, 3.70 mmol), and TMSCl (0.51 mL, 4.07 mmol).

3,4-Dichloro-phenyl-biguanide (3f)

Compound 3f was obtained according to the general procedure, as a beige powder from dicyandiamide 2 (518.77 mg, 6.17 mmol), 3,4-dichloroaniline 1f (1 g, 6.17 mmol) and TMSCl (0.86 mL, 6.79 mmol).

General procedure for the synthesis of 2,4-diamino-1,3,5-triazines

To a solution of corresponding biguanide, 3 (1 eq) in 15 ml of anhydrous methanol (MeOH) cooled at 0°C under nitrogen, were added sodium methanolate or sodium ethanolate (10 to 15 eq). To the cooled mixture, the corresponding ester derivative 4 (1.3 to 1.5 eq) was added. The reaction mixture was brought to room temperature and stirring was maintained for 30 min. Heating was conducted at reflux for 3h to 8h, depending on the nature of biguanide derivative 3. After refluxing, the mixture was cooled in ice crushed and the precipitate formed was filtered. The product was purified either by extraction or washing in organic solvent recrystallization or column chromatography. After purification, the 2,4-diamino-1,3,5-triazine derivatives (5a-k) were obtained as colored powders with yields from 16% to 86%.

6-(4-chlorophenyl)-N2-(4-(methylthio)phenyl)-1,3,5-triazine-2,4-diamine (5a)

Compound 5a was synthesized according to the general procedure from 4-methylthio-phenyl-biguanide hydrochloride 3a (1.05 g, 4.70 mmol), sodium methanolate (3 mL, 53.3 mmol), and 4-chlorobenzoate 4a (0.87 mL, 5.56 mmol). The precipitate formed was washed with dichloromethane (DCM), dried, and obtained as a beige powder with a yield of 16%. Mp 168 °C. 1H NMR (400 MHz, DMSO-d6), δ: 2.45 (s, 3H, SCH3), 7.24 (d, J = 8.3 Hz, 4H, H-2'', H-6'', Nh2), 7.58 (d, J = 8.4 Hz, 2H, H-3'', H-5''), 7.79 (d, J = 8.1 Hz, 2H, H-3', H-5'), 8.30 (d, J = 8.3 Hz, 2H, H-2', H-6'), 9.60 (s, 1H, NH). 13C NMR (101 MHz, DMSO):15.89 (SCH3), 120.69 (C-2'', C-6''), 127.29 (C-2', C-6'), 128.40 (C-3', C-5'), 129.49 (C-4''), 130.33 (C-3'', C-5''), 135.61 (C-1'), 136.13 (C-4'), 137.49 (C-1''), 164.45 (C-4), 167.08 (C-6), 169.20 (C-2). IR, (cm-1): 3317.56 (N-H), 1361.74 (CH3), 1658.78 (C=Car), 802.39 (C-Har). UPLC ESI/MS, [M+H]+: 344.8.

6-(4-chlorophenyl)-N2-(2-morpholinoethyl)-1,3,5-triazine-2,4-diamine (5b)

Compound 5b was synthesized according to the general procedure from 4-ethylmorpholinyl-biguanide hydrochloride 3b (1 g, 3.99 mmol), sodium methanolate (3 mL, 53.3 mmol) and 4-chlorobenzoate 4a (0.75 mL, 4.79 mmol). The reaction mixture was cooled, filtered, and evaporated under vacuum. The residue was put in water and extracted several times with diethyl ether. The organic phase was dried and evaporated to yield the expected product as a white powder with a yield of 34%. Mp 187 °C. 1H NMR (400 MHz, DMSO-d6), δ: 2.44 (m, 6H, H-2', H-3''', H-5'''), 3.36 - 3.50 (m, 2H, H-1'), 3.51 - 3.60 (m, 4H, H-2''', H-6'''), 6.80 (br s, 1H, NH), 6.93 (s, 2H, Nh2), 7.54 (m, 2H, H-3'', H-5''), 8.25 (m, 2H, H-2'', H-6''). 13C NMR (101 MHz, DMSO-d6), δ: 53.29 (C-1'), 57.41 (C-3''', C-5''', C-2'), 66.17 (C-2'', C-6''), 128.23 (C-2'', C-6''), 129.34 (C-3'', C-5''), 135.75 (C1''), 135.88 (C-4''), 166.00 (C-6), 167.23 (C-4), 168.55 (C-2). IR, *v*(cm-1): 3267.41 (N-H), 2976.16 (C=CH2), 1604.77 (C=Car), 813.96 (C-Har). UPLC ESI/MS, [M+H]+: 335.8.

4-((4-amino-6-(4-chlorophenyl)-1,3,5-triazin-2-yl)amino)benzonitrile (5c)

Compound 5c was synthesized according to the general procedure from 4-cyanophenyl-biguanide hydrochloride 3c (1.05 g, 5.2 mmol), sodium methanolate (3 mL, 53.3 mmol), and 4-chlorobenzoate 4a (0.84 mL, 5.37 mmol). The precipitate formed was washed with water, and methanol and recrystallized from MeOH/H2O mixture (50/50). After filtration, the precipitate was dried and obtained as a white powder with a yield of 30%. Mp 298 °C. 1H NMR (400 MHz, DMSO-d6), δ : 7.42 (br s, 2H, NH2), 7.54 - 7.66 (m, 2H, H-2'', H-6''), 7.75 (d, J = 8.8 Hz, 2H, H-3'', H-5''), 8.06 (d, J = 8.8 Hz, 2H, H-3', H-5'), 8.24 - 8.37 (m, 2H, H-2', H-6'), 10.11 (s, 1H, NH), 13C NMR (101 MHz, DMSO-d6), δ : 103.24 (C-4''), 119.41 (CN), 119.48 (C-2'', C-6''), 128.49 (C-2', C-6'), 129.57 (C-3', C-5'), 132.84 (C-3'', C-5''), 135.29 (C-1'), 136.39 (C-4'), 144.4 (C-1''), 164.51 (C-6), 167.06 (C4), 169.54 (C-2). IR, *v*(cm-1): 3317.56 (N-H), 1662.64 (C=Car), 802.39 (C-Har). UPLC ESI/MS, [M+H]+: 323.8.

N2-(benzo[d][1,3]dioxol-5-yl)-6-(4-chlorophenyl)-1,3,5-triazine-2,4-diamine (5d)

Compound 5d was synthesized according to the general procedure from benzo[d][1,3]dioxol-5-biguanide hydrochloride 3d (800 mg, 3.62 mmol), sodium methanolate (3 mL, 53.3 mmol) and 4-chlorobenzoate 4a (85 mL, 5.43 mmol). The precipitate formed was successively washed with methanol, water, and diethyl ether and dried. It was obtained as a green powder with a yield of 52%. Mp 206 °C. 1H NMR (400 MHz, DMSO-d6), δ: 5.98 (s, 2H, OCH2O), 6.85 (d, J = 8.4 Hz, 1H, H-5'), 7.14 (m, 3H, NH2, H-2'), 7.58 (m, 3H, H-3'', H-5'', H-6'), 8.28 (d, J = 8.4 Hz, 2H, H-2'', H-6''), 9.48 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ: 100.75 (OCH2O), 102.48 (C-2'), 107.77 (C-5'), 112.80 (C-6'), 128.38 (C-2'', C-6''), 129.42 (C-3'', C-5''), 134.13 (C-1''), 135.68 (C-4''), 136.07 (C-1'), 142.21 (C-4'), 146.91 (C-3'), 164.38 (C-6), 167.05 (C-4), 169.09 (C-2). IR, *v*(cm-1): 3325.28 (NH), 1658 (C=Car), 1473.62 (C=CH2), 808.17 (C-Har). UPLC ESI/MS, [M+H]+: 342.8.

6-(4-chlorophenyl)-N2-(2,4-dichlorophenyl)-2,4-diamino-1,3,5-triazine (5e)

Compound 5e was synthesized according to the general procedure from 2,4-dichloro-phenyl-biguanide hydrochloride 3e (450 mg, 1.83 mmol), sodium methanolate (3 mL, 53.3 mmol) and 4-chlorobenzoate 4a (0.43 mL, 2.74 mmol). The precipitate formed was successively washed with methanol, water, and cyclohexane and dried. It was obtained as a white powder with a yield of 40%. Mp. 239 °C. 1H NMR (400 MHz, DMSO-d6), δ: 7.18 (br s, 2H, NH2), 7.44 (dd, J = 8.7, 2.4 Hz, 1H, H-6''), 7.56 (d, J = 8.6 Hz, 2H, H-3', H-5'), 7.68 (d, J = 2.4 Hz, 1H, H-5''), 7.78 (d, J = 8.7 Hz, 1H, H-3''), 8.23 (d, J = 8.5 Hz, 2H, H-2', H-6'), 8.97 (s, 1H, NH). 13C NMR(101 MHz, DMSO-d6), δ: 127.37 (C-6''), 128.48 (C-4''), 128.58 (C-2''), 128.79 (C-5''), 129.16 (C-2', C-6'), 129.48 (C-3', C-5'), 129.59 (C-3''), 134.95 (C-1'), 135.36 (C-4'), 136.21 (C-1''), 165.11(C-6), 167.21 (C-4), 169.33 (C-2). IR, *v*(cm-1): 3342.64 (N-H), 1591.27 (C=Car, 804.32 (C-Har). UPLC ESI/MS, [M+H]+: 367.6.

6-(4-chlorophenyl)-N2-(3,4-dichlorophenyl)-2,4-diamino-1,3,5-triazine (5f)

Compound 5f was synthesized according to the general procedure from 3,4-dichloro-phenyl-biguanide hydrochloride 3f (500 mg, 2.03 mmol), sodium methanolate (3 mL, 53.3 mmol) and 4-chlorobenzoate 4a (0.48 mL, 3.045 mmol). The precipitate formed was washed with methanol, water, and cyclohexane and dried. It was obtained as a white powder with a yield of 45%. Mp. 252°C 1H NMR (400 MHz, DMSO-d6), δ: 7.37 (sl, 2H, NH2), 7.54 (d, J = 8.8 Hz, 1H, H-2''), 7.60 (d, J = 8.5 Hz, 2H, H-3', H-5'), 7.80 (dd, J = 8.8, 2.3 Hz, 1H, H-2''), 8.22 (d, J = 1.8 Hz, 1H, H-5''), 8.29 (d, J = 8.5 Hz, 2H, H-2', H-6'), 9.90 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ : 119.67 (C-6''), 120.78 (C-2''), 123.23 (C-4''), 128.47 (C-2', C-6'), 129.48 (C-3', C-5'), 130.18(C-5''), 130.72 (C-3''), 135.37 (C-1'), 136.33 (C-4'), 140. 14 (C-1''), 164.39 (C-6), 167.02 (C-4), 169.39 (C-2).IR, *v*(cm-1): 3342.64 (N-H), 1591.27 (C=

= Car), 804.32 (C-Har). UPLC ESI/MS, [M+H]+: 367.6.

N-2-(4-(methylthio)phenyl)-6-(pyridin-2-yl)-1,3,5-triazine-2,4-diamine (5g)

Compound 5g was synthesized according to the general procedure from 4-thiomethyl-phenyl-biguanide hydrochloride 3g (525 mg, 2.35 mmol), sodium ethanolate (3 mL, 38.26 mmol), and ethyl-2-picolinate 4b (0.41 mL, 3.05 mmol). The reaction mixture was evaporated and the solid obtained was recrystallized from a MeOH/H2O mixture (1/2). It was obtained as a beige powder with a yield of 23%. Mp 172 °C. 1H NMR (400 MHz, DMSO-d6), δ : 2.45 (s, 3H, CH3), 7.26 (m, 4H, H-2', H-6', NH2), 7.54 (m, 1H, H-4''), 7.83 (d, J = 8.7 Hz, 2H, H-3', H-5'), 7.96 (td, J = 7.7, 1.7 Hz, 1H, H-5''), 8.26 (d, J = 7.9 Hz, 1H, H-6''), 8.71 (d, J = 4.6 Hz, 1H, H3''), 9.78 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ : 15.91 (CH3), 120.50 (C-2', C-6'), 123.23 (C-4''), 125.41 (C-6''), 127.31 (C-4'), 130.17 (C-3', C-5'), 136.70 (C-1'), 137.63 (C-5''), 149.23 (C-3''), 154.34 (C-1''), 164.67 (C-6), 167.33 (C-2), 170.17 (C-4). IR, *v*(cm-1): 3180.62 (N-H), 1606.70 (C=Car), 1369.46 (CH3), 825.33 (C-Har). UPLC ESI/MS, [M+H]+: 311.4

N2-(2,4-dichlorophenyl)-6-(pyridin-2-yl)-2,4-diamino-1,3,5-triazine (5h)

Compound 5h was synthesized according to the general procedure from 2,4-dichloro-phenyl-biguanide hydrochloride 3h (400 mg, 1.625 mmol), 3 mL of sodium ethanolate (38.26 mmol) and ethyl-2-picolinate 4b (0.44 mL, 3.25 mmol). The reaction mixture was evaporated. Then, the solid formed was purified on column chromatography with a DCM/MeOH mixture (9/1). After evaporation under vacuum, the dry residue was washed with DCM and was obtained as a pink powder with a yield of 45%. Mp 230 °C. 1H NMR (400 MHz, DMSO-d6), δ: 7.02-8.04 (m, 7H, H-3', H-5', H-6', H-4'', H-5'', NH2), 8.20 (d, J = 7.7 Hz, 1H, H-6''), 8.69 (d, J = 3.8 Hz, 1H, H-3''), 9.07 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ: 123.31 (C-6'), 125.47 (C-4''), 127.39 (C-6''), 128.50 (C-4'), 128.80 (C-5'), 129.23 (C-2'), 135.00 (C-3'), 136.72 (C-1'), 149.25 (C-5''), 149.25 (C-3''), 154.17 (C1''), 165.38 (C-6), 167.50 (C-2), 170.40 (C-4). IR, *v* (cm-1): 3113.11 (N-H), 1606,70 (C=Car), 812,03 (C-Har). UPLC ESI/MS, [M+H]+: 334.2.

N2-(3,4-dichlorophenyl)-6-(pyridin-2-yl)-2,4-diamino-1,3,5-triazine (5i)

Compound 5i was synthesized according to the general procedure from 3,4-dichloro-phenyl-biguanide hydrochloride 4i (510 mg, 2.07 mmol), 3 mL of sodium ethanolate (38.26 mmol), and ethyl-2-picolinate 4b (0.56 mL, 4.14 mmol). The precipitate formed was successively washed with methanol, water, and diethyl ether and dried. It was obtained as a pink powder with a yield of 86%. Mp 243 °C. 1H NMR (400 MHz, DMSO-d6), δ: 7.227.67 (m, 4H, H-2', H-5', NH2), 7.84 (d, J = 8.5 Hz, 1H, H-4''), 7.98 (t, J = 7.2 Hz, 1H, H-6'), 8.29 (m, 2H, H-5'', H-6''), 8.72 (d, J = 3.3 Hz, 1H, H-3''), 10.11 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ: 119.55 (C-6'), 120.66 (C-2'), 123.18 (C-4'), 123.27 (C-4''), 125.67 (C-6''), 130.16 (C-5'), 130.75 (C-3'), 136.88 (C-5''), 140.23 (C-1'), 149.36 (C-3''), 153.94 (C-1''), 164.63 (C-6), 167.19 (C-2), 170.20 (C-4). UPLC ESI/MS, [M+H]+: 334.03. IR, *v*(cm-1): 3147.83 (N-H), 1598.99 (C=Car), 790.81 (C-Har). UPLC ESI/MS, [M+H]+: 334.2.

N2-(benzo[d][1,3]dioxol-5-yl)-6-(pyridin-4-yl)-2,4-diamino-1,3,5-triazine (5j)

Compound 5j was synthesized according to the general procedure from benzo[d][1,3]dioxol-5-biguanide hydrochloride 4j (650 mg, 2.94 mmol), sodium ethanolate (3 mL, 38.26 mmol) and ethyl isonicotinate 4c (0.57 mL, 3.822 mmol). The precipitate formed was successively washed with methanol, water and DCM then dried. It was obtained as a grey powder with a yield of 24%. Mp 218 °C. 1H NMR (400 MHz, DMSO-d6), δ: 5.99 (s, 2H, Ch2), 6.86 (d, J = 8.4 Hz, 1H, Hb), 7.15 (dd, J = 8.4, 1.8 Hz, 1H, Ha), 7.31 (s, 2H, NH2), 7.58 (s, 1H, He), 8.11 (d, J = 5.5 Hz, 2H, Hg), 8.76 (d, J = 5.7 Hz, 2H,

Hh), 9.60 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ: 100.79 (OCH2O), 102.59 (C-2'), 107.79 (C-5'), 112.97 (C-6'), 121.46 (C-2'', C-6''), 133.93 (C-1'), 142.37 (C-1''), 144.21 (C-3''), 146.93 (C-5''), 150.18 (C-6), 164.42 (C-4), 167.13 (C6), 168.64 (C4). IR, *v*(cm-1): 3180.62 (N-H), 1591.27 (C=Car), 1487.12 (C=CH2), 802.39 (C- Har). UPLC ESI/MS, [M+H]+: 309.10.

N2-(3,4-dichlorophenyl)-6-(pyridin-4-yl)-1,3,5-triazine-2,4-diamine (5k)

Compound 5k was synthesized according to the general procedure from 1-(3,4-dichlorophenyl)biguanide hydrochloride 3k (500 mg, 2.03 mmol), sodium ethanolate (3 mL, 38.26 mmol), and ethyl isonicotinate 4c (0.39 mL, 2.64 mmol). The precipitate formed was successively washed with methanol, water, and diethyl ether and then dried. It was obtained as a pink powder with a yield of 36%. Mp 276 °C. 1H NMR (400 MHz, DMSO-d6), δ: 7.55 (m, 3H, H-2', NH2), 7.81 (dd, J = 8.9, 2.4 Hz, 1H, H-5'), 8.12 (d, J = 5.9 Hz, 2H, H-2'', H-6''), 8.22 (d, J = 1.7 Hz, 1H, H-6'), 8.77 (d, J = 5.8 Hz, 2H, H-3'', H-5''), 10.01 (s, 1H, NH). 13C NMR (101 MHz, DMSO-d6), δ: 119.81 (C-6'), 120.92 (C-2'), 121.49 (C-6'', C-2''), 123.47 (C-4'), 130.23 (C-5'), 130.75 (C-3'), 139.98 (C-1'), 143.91 (C-1''), 150.27 (C-3'', C-5''), 164.46 (C-6), 167.11 (C-4), 168.96 (C-2). IR, *v*(cm-1): 3307.92 (N-H), 1600.32 (C=Car), 804.32 (C-Har). UPLC ESI/MS, [M+H]+: 334.03.

RESULTS AND DISCUSSION

Chemistry

In this work, novel triazines were synthesized in two steps. The first step consisted of adding primary amines 1 with dicyandiamide 2 in the presence of chlorotrimethylsilane in acetonitrile, under microwave irradiation for 10 min at 130 °C (18 bar, 60 W). The intermediates resulting from this first step were biguanides 3a-f **(Figure 1).**

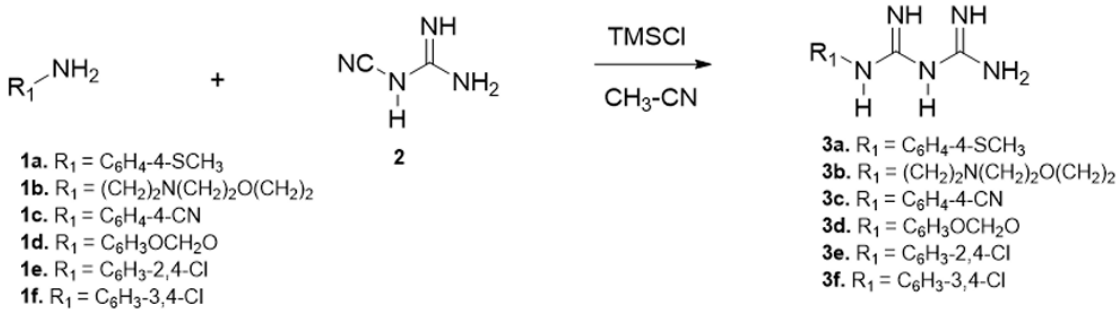
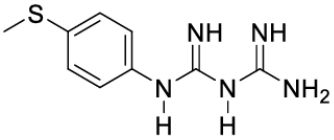
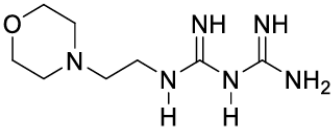
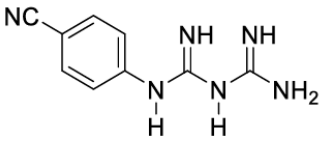
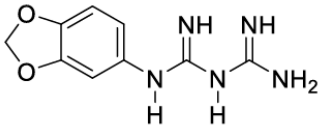
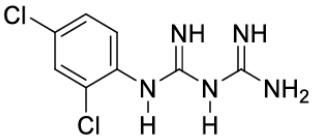
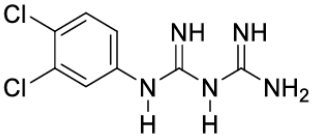


Figure 1. Synthesis of biguanides

At the end of the reaction, the biguanide formed was cooled in ice crushed, filtered, and washed with acetonitrile and isopropanol. The resulting biguanide in powdery form was dried and used without further purification for the next step [18]. The yields of synthesized biguanides ranged from 61-95% **(Table 1).**

Table 1. Synthesized biguanides.

Molecules	Structures	Yields (%)
3a		61
3b		95
3c		92
3d		76
3e		70
3f		50

Then, biguanides previously obtained were condensed with various esters of interest according to the reaction scheme **(Figure 2).**

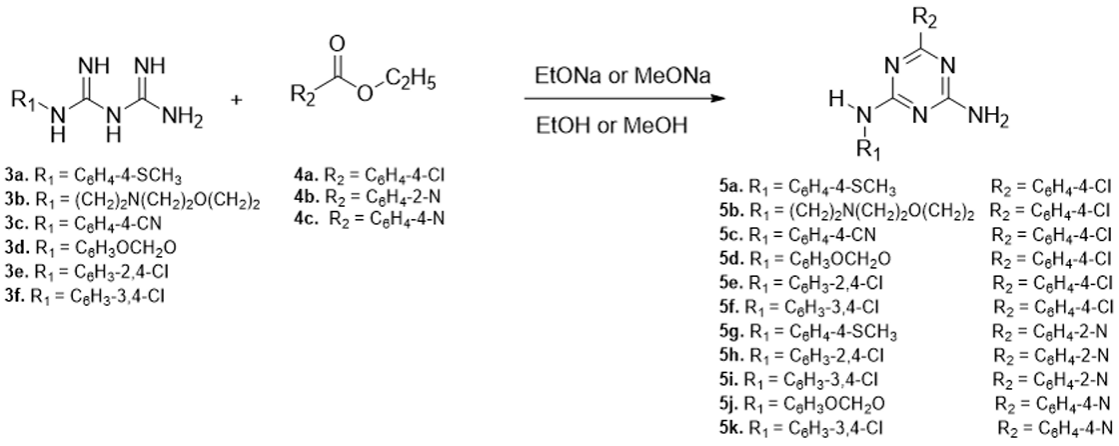
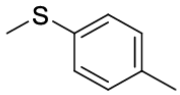
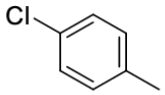
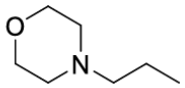
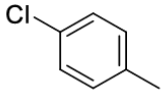
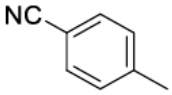
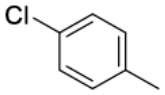
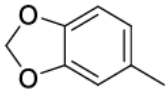
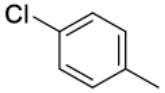
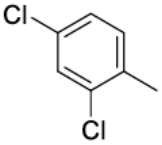
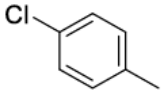
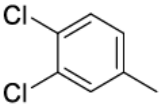
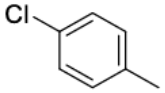
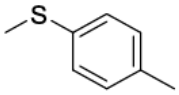
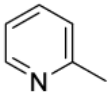
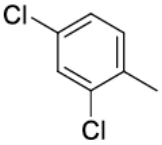
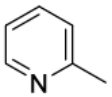
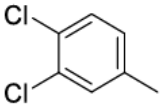
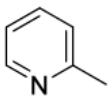
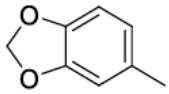
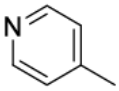
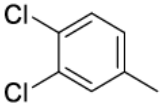
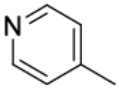


Figure 2. Synthesis of 6-aryl-2,4-diamino-1,3,5-triazines

The reaction was carried out under an inert atmosphere, at the reflux of ethanol or methanol according to the chosen base (EtONa or MeONa [19]). The reaction was followed by TLC and UPLC-MS. After completion of the reaction, synthesized triazines 5a-k precipitated. The precipitate obtained was recovered by filtration and washed successively with methanol and dichloromethane. Depending on its

solubility, it was rinsed with ethyl acetate, diethyl ether, or cyclohexane. If necessary, it was either recrystallized in a methanol/water mixture 1:1 or 1:2 or purified on a silica gel column with a convenient solvent. The yields of 6-aryl-2,4-diamino-1,3,5-triazines (5a-k) ranged from 16-86% **(Table 2).**

Table 2. Synthesized triazines.

Molecules	R_1	R_2	Yields (%)
5a			16
5b			34
5c			30
5d			52
5e			40
5f			45
5g			23
5h			45
5i			86
5j			24
5k			36

Biological section

Table 3. Experimental conditions used for protein kinase assays.

Protein Kinase (Family)	Enzyme Description	Substrate* (Working concentration)	Buffer used**
GSK3α (CMGC)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	GS-1 peptide: YRRAAVPPSPSLSRHSSPHQSpEDEEE *** (20 µM)	**A**
GSK3β (CMGC)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	GS-1 peptide: YRRAAVPPSPSLSRHSSPHQSpEDEEE *** (20 µM)	**A**
CDK5/p25 (CMGC)	Human, recombinant, expressed in bacteria	Histone H1 (37.2 µM)	**A**
EGFR (TK)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Poly(L-glutamic acid – L-tyrosine) sodium salt (0.17 µg/µL)	**A**
VEGFR2 (TK)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Poly(L-glutamic acid – L-tyrosine) sodium salt (0.17 µg/µL)	**A**
EphB1 (TK)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Poly(L-glutamic acid – L-tyrosine) sodium salt (0.17 µg/µL)	**A**
JAK3 (TK)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Peptide: GGEEEEYFELVKKKK (94 µM)	**A**
ABL1 (TK)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Peptide: EAIYAAPFAKKK (127 µM)	**A**
DYRK1A (CMGC)	Human recombinant, expressed by baculovirus in Sf9 insect cells	Peptide: KKISGRLSPIMTEQ (10.7 µM)	**A**
CLK1 (CMGC)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Peptide: GRSRSRSRSRSR (57.3 µM)	**A**
CK1ε (CK1)	Human, recombinant, expressed by baculovirus in Sf9 insect cells	Peptide: RRKHAAIGSpAYSITA *** (170 µM)	**A**
PIM1 (CAMK)	Human proto-oncogene, recombinant, expressed in bacteria	PimTide: ARKRRRHPSGPPTA (630 µM)	**A**

* Peptide substrates were obtained from ProteoGenix (Schiltigheim, France) or Sigma for Histone H1, Poly (L-glutamic acid – L-tyrosine) sodium salt

** Composition of the buffers: Buffer A: 10 mM MgCl2, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 µg/mL heparin *** "Sp" stands for phosphorylated serine

Primary screening and IC50 determination

In this study, synthesized triazines were tested on 12 protein kinases (GSK3-α/β, CDK5/p25, EGFR, VEGFR2, EphB1, JAK3, ABL1, DYRK1A, CLK1, CK1ε, and PIM1) (Table 3) to evaluate their potential enzymatic activity. Kinase activities were measured using the ADP-GloTM bioluminescent kinase assay kit (Promega, Madison, WI) according to the recommendations of the manufacturer, described according to the protocol of Zegzouti et al. [20]. These enzymatic activities were detected in the presence of 10 µM ATP. Reactions were carried out in the presence of 10 µM of ATP, in a final volume of 6 µl for 30 min at 30 °C in ADP-Glo buffer (25 mM Tris-HCl pH 7.5, 10 mM MgCl2, 1 mM EGTA, 1 mM DTT, 50 µg/mL heparin and 0.1 mg/ml of BSA). After that, 6 µl of ADP-GloTM Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature, 12 µl of ADP-GloTM Kinase Detection Reagent was added for one hour at room temperature. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate

luminometer and expressed in Relative Light Unit (RLU). Kinase activities were expressed in % of maximal activity, i.e. measured in the absence of an inhibitor. In primary screening, chemical compounds, dissolved in DMSO, are tested at 10 µM and 1 µM final in the kinase assay. To determine the half-maximal inhibitory concentration (Ic50), the assays were performed in duplicate in the absence or presence of increasing doses of the tested compounds. Positive controls (for total activity) and negative controls (for background noise) were performed with appropriate dilutions of dimethylsulfoxide (DMSO). Note that a value ≥ 100 indicates that the compound tested can not inhibit the enzymatic activity of the kinase at the concentration tested **(Table 4).**

Table 4. Primary screening of triazines.

Compound	Concentration	$GSK3_{\alpha}$	$GSK3_{\beta}$	CDK5/p25	EGFR	VEGFR2	EphB1	JAK3	ABL1	DYRK1A	CLK1	CK1ε	PIM1
5a	10 µM	90	86	89	≥ 100	≥ 100	**42**	55	≥ 100	85	71	55	**43**
	1 µM	≥ 100	87	≥ 100	89	≥ 100	70	71	≥ 100	102	99	≥ 100	≥ 100
5b	10 µM	≥ 100	≥ 100	≥ 100	≥ 100	78	≥ 100	≥ 100	≥ 100	92	65	≥ 100	**27**
	1 µM	≥ 100	≥ 100	89	≥ 100	85	90	≥ 100	≥ 100	≥ 100	100	≥ 100	55
5c	10 µM	100	≥ 100	67	79	96	**48**	**18**	≥ 100	86	67	97	74
	1 µM	97	97	≥ 100	100	100	91	81	≥ 100	92	99	≥ 100	97
5d	10 µM	68	71	67	70	66	**44**	59	87	90	71	44	46
	1 µM	83	85	87	78	70	74	59	86	≥ 100	≥ 100	93	≥ 100
5e	10 µM	94	89	96	≥ 100	≥ 100	95	77	86	≥ 100	≥ 100	87	62
	1 µM	100	82	99	≥ 100	≥ 100	73	86	74	98	≥ 100	≥ 100	≥ 100
5f	10 µM	≥ 100	91	86	97	≥ 100	**48**	74	57	≥ 100	≥ 100	90	82
	1 µM	≥ 100	91	90	97	≥ 100	61	51	50	96	≥ 100	100	≥ 100
5g	10 µM	98	80	≥ 100	90	≥ 100	**29**	76	77	91	≥ 100	78	77
	1 µM	≥ 100	81	98	99	≥ 100	66	78	73	92	≥ 100	95	≥ 100
5h	10 µM	79	78	83	81	72	53	70	60	≥ 100	98	97	78
	1 µM	78	96	≥ 100	70	62	56	73	53	≥ 100	≥ 100	110	99
5i	10 µM	76	≥ 100	≥ 100	69	50	55	83	56	≥ 100	97	93	86
	1 µM	66	60	≥ 100	70	51	70	**45**	54	≥ 100	≥ 100	≥ 100	≥ 100
5j	10 µM	68	85	68	74	49	73	57	58	72	51	71	≥ 100
	1 µM	96	≥ 100	≥ 100	93	80	82	76	87	96	89	≥ 100	≥ 100
5k	10 µM	96	97	83	≥ 100	91	58	≥ 100	124	**49**	50	57	77
	1 µM	≥ 100	93	≥ 100	≥ 100	104	**43**	≥ 100	99	62	89	90	89

Primary screening results highlighted seven compounds 5a, 5b, 5c, 5d, 5g, 5i and 5k, which inhibited kinases EphB1, JAK3, CK1Ɛ, and PIM1. Enzymatic activity percentages of these compounds, at 10 and 1 µM concentrations, were noticed in **Table 4.** Dose-response studies were subsequently undertaken with Ic50 determination. IC50 of active molecules which obtained the best results were determined in **Table 5.**

Table 5. IC50 determination at 10 µM for active triazines

Compounds	IC_{50} (µg/mL)		
	EphB1	PIM1	JAK3
5a	3.07	-	-
5b	-	1.18	-
5c	-	-	1.56
5d	-	1.76	-
5g	15.43	-	-

From these different results, we were able to identify two chemical cores favorable to the inhibition of enzymatic activity. These are 6-chlorophenyl-1,3,5-triazine and 6-(pyridin-2-yl)-1,3,5-triazine which carry an aminophenyl group in position 2. The latter must be monosubstituted in the para position of amine function by a thiomethyl or nitrile group (compounds 5a, 5c, and 5g). On the one hand, polysubstitution in the ortho and para position or the meta and para position by chlorine atoms led to inactivity on kinases. On the other hand, introduction into position 2, on 6-chlorophenyl-2,4-diamino-1,3,5-triazine of ethylamino-morpholinyl substituent (compound 5b) or 5aminobenzodioxole (compound 5d) caused inhibition of protein kinases. Moreover, the most active molecule was compound 5b which inhibited PIM1 kinase (IC50 = 1.18 µg/mL), Table 5. However, introduction at position 6 of the pyridine-4-yl group on 1,3,5-triazine moiety resulted in a lack of enzymatic activity, even though this structural variation was coupled with the presence, in position 2, of the group previously favorable to inhibition such as 5aminobenzodioxole (5j).

CONCLUSION

Here, we have reported the synthesis in two steps and the biological evaluation of a series of new triazines 5a-k. We successfully developed a library of 11 new compounds with overall yields ranging from 16-86%. For the first step, we used microwave irradiation for the synthesis of biguanides 3a-e. This approach allowed structural diversity of intermediates biguanides 3a-e which led to final triazines. Enzymatic inhibition was measured on all final compounds. Results on protein kinase assays showed that the most active molecule was compound 5b which inhibited PIM1 kinase with the value of IC50 = 1.18 µg/mL. This result is the starting point of a larger research program within our group that can investigate the introduction of substituted groups on triazine's terminal amino group. Therefore, the perspectives identified will concern the search for new pharmaco-modulations and structural optimization favorable to the increase of protein kinases inhibitory activity involved in immune pathophysiology, inflammatory or cancerous diseases, to generate lead compounds, and be able to conduct studies on relevant in vivo models.

ACKNOWLEDGMENTS: We would like to thank Cancéropôle Grand Ouest for its support for this work as well as the different teams of the network with whom we have had constructive exchanges.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: This work was financially supported by the Cooperation and Cultural Action Service of the French Embassy in Côte d'Ivoire (SCAC).

ETHICS STATEMENT: None

REFERENCES

1. Reys V, Labesse G. In silico profiling of protein kinase inhibitors. Med Sci. 2020;36:38-41.
2. Wilson LJ, Linley A, Hammond DE, Hood FE, Coulson JM, MacEwan DJ, et al. New perspectives, opportunities, and challenges in exploring the human protein kinome. Cancer Res. 2018;78(1):15-29.
3. Quesada GY. The regulation of Staufen1 in cell cycle and proliferation [PhD thesis]. Department of Biochemistry and Molecular Medicine, Faculty of Medicine, University of Montreal. 2023, 1-211.
4. El Jammal T, Gerfaud-Valentin M, Seve P, Jamilloux Y. Inhibitors of JAK/STAT signaling during rheumatological diseases: A growing spectrum. Rheum Rev. 2020;87(4):261-72.
5. Sailliet N, Brosseau C, Robert JM, Brouard S. Role of JAK inhibitors and immune cells in transplantation. Cytokine Growth Factor Rev. 2019;47:62-73.
6. Ayala-Aguilera CC, Valero T, Lorente-Macías Á, Baillache DJ, Croke S, Unciti-Broceta A. Small molecule kinase inhibitor drugs (1995–2021): Medical indication, pharmacology, and synthesis. J Med Chem. 2022;65(2):1047-131.
7. Geier CB, Sauerwein KMT, Leiss-Piller A, Zmek I, Fischer MB, Eibl MM, et al. Hypomorphic mutations in the BCR signalosome lead to selective immunoglobulin M deficiency and impaired B-cell homeostasis. Front Immunol. 2018;9:2984.
8. Chen X, Jin J, Wang Q, Xue H, Zhang N, Du Y, et al. A de novo pathogenic CSNK1E mutation identified by exome sequencing in family trios with epileptic encephalopathy. Hum Mutat. 2019;40(3):281-7.
9. Narasimamurthy R, Hunt SR, Lu Y, Fustin JM, Okamurae H, Partch CL, et al. CK1δ/ε protein kinase primes the PER2 circadian phosphoswitch. Proc Natl Acad Sci USA. 2018;115(23):5986-91.
10. Abramov D, Guiberson NGL, Burré J. STXBP1 encephalopathies: Clinical spectrum, disease mechanisms, and therapeutic strategies. J Neurochem. 2021;157(2):165-78.
11. McInnes IB, Gravallese EM. Immune-mediated inflammatory disease therapeutics: Past, present and future. Nat Rev Immunol. 2021;21(10):680-6.
12. David T, Ling SF, Barton A. Genetics of immune-mediated inflammatory diseases. Clin Exp Immunol. 2018;193(1):3-12.
13. Yan W, Zhao Y, He J. Anti-breast cancer activity of selected 1,3,5-triazines via modulation of EGFR-TK. Mol Med Rep. 2018;18(5):4175-84.
14. Junaid A, Lim FPL, Chuah LH, Dolzhenko AV. N2 -Diaryl-1,3,5-triazine-2,4-diamines: Synthesis, antiproliferative activity, and 3D-QSAR modeling. RSC Adv. 2020;10(21):12135-44.
15. Gucev Z, Tasic V, Plaseska-Karanfilska D, Dimishkovska M, Laban N, Bozinovski Z, et al. Severe digital malformations in a rare variant of fibrodysplasia ossificans progressiva. Am J Med Genet A. 2019;179(7):1310-4.

16. Kawahata W, Asami T, Kiyoi T, Irie T, Taniguchi H, Asamitsu Y, et al. Design and synthesis of novel aminotriazine analogs as selective Bruton's tyrosine kinase inhibitors for the treatment of rheumatoid arthritis. J Med Chem. 2018;61(19):8917-33.
17. Díaz-Ortiz Á, Elguero J, Foces-Foces C, De La Hoz A, Moreno A, Del Carmen Mateo M, et al. Green synthesis and self-association of 2,4-diamino-1,3,5-triazine derivatives. New J Chem. 2004;28(8):952-8.
18. Nagasawa H, Narise K, Okuda K, Hirayama T, Enomoto Y. Optimization of biguanide derivatives as selective antitumor agents blocking adaptive stress responses in the tumor microenvironment. Drug Des Dev Ther. 2014:701-17.
19. Tsitsa P, Antoniadou-Vyza E, Hamodrakas S, Eliopoulos E, Tsantili-Kakoulidou A, Lada-Hytiroglou E, et al. Synthesis, crystal structure, and biological properties of a new series of lipophilic s-triazines, dihydrofolate reductase inhibitors. Eur J Med Chem. 1993;28(2):149-58.
20. Zegzouti H, Zdanovskaia M, Hsiao K, Goueli SA. ADP-Glo: A Bioluminescent and homogeneous ADP monitoring assay for kinases. Assay Drug Dev Technol. 2009;7(6):560-72.

Efficacy of Homoeopathic Medicines in LM Potency for Treating Hypothyroidism

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ABSTRACT

Hypothyroidism is a clinical Syndrome resulting from a deficiency of thyroid hormones, which leads to generalized slowing down of metabolic processes. In infants and children results in marked slowing of growth &development with mental retardation. Whereas in adults leading to slowed heart rate, diminished oxygen consumption & deposition of glycosaminoglycans in intracellular spaces particularly in skin & muscles, female is more prone to suffer from this disorder. The risk is multiplied with increasing age and family history of thyroid disorder. The conventional system deals with prolonged use of Levothyroxine with subsequent increase or decrease of its doses depending on its condition. In homoeopathy we treat them with forming the Totality of Symptoms and by individualistic approach. I report a case of newly Married lady of 24 years old presented with hairfall, mood swings, fatigue and Menstrual irregularities. Natrum Muraticum in 50 millesimal(LM)potency was prescribed on the basis of totality and individualization.

Key words: Homoeopathy, LM Potency, Endocrine disorder, Hypothyroidism

INTRODUCTION

Hypothyroidism is a clinical condition due to lack of production of thyroid hormones for prolonged period [1]. The clinical presentations depend on age of its onset and severity. Cretinism in infancy and childhood & myxoedema in adulthood [2]. It can be primary hypothyroidism due to the intrinsic disorder of thyroid gland. When a middle-aged woman presents with vague symptoms like fatigue, weight gain, or depression, a high index of suspicion should be maintained for diagnosis because secondary hypothyroidism, which results from failure of TSH production due to diseases of the anterior pituitary or hypothalamus, is typically not clinically obvious [3, 4]. m instances of cretiny A Feature A decrease in T3 and T4 levels and an increase in TSH levels are among the laboratory results. Although instances with suprathyroid lesions have low TSH levels, myxoedema is diagnosed by low blood T3 and T4 levels with noticeably high TSH [5]. In India, it is the most prevalent thyroid condition, affecting one out of ten persons.

Case report

A newly married lady of 24 years old visited my clinic with complaints of severe hair fall for last 9 months, with mood swings, fatigue and irregular menstruation also experienced from that particular period. She was already a diagnosed case of hypothyroidism and was under Thyronorm 50mg and her doctor advised her 75mg on 14.06. 2022.then the couple decide to try homoeopathy after some recommendations. She suffered with pneumonia at the age of 3. Her mother suffered from hypothyroidism. Grand father and mother (paternal) suffered from hypertension, Urine 7-8 times during day at least 1-2 times in night with burning Menarche at the age of 16. Irregular menstruation with 35-37 days'cycle. Clots, Headache on sun exposure, dry skin with itching, Sweating more on scalp and face, generally not comfortable in warmth.

Physical generals
She was whitish complexioned, Average built
Preferred Spicy food++++and pizza+ or even fried food+
Frequent Urination with burning
Menarche at the age of 16. Menstruation irregular with 35-37 days' cycle, clots, Headache on sun exposure.dry
skin with itching, Sweating more on scalp and face, generally not comfortable in warmth [5].

Mentals
The Lady looked calm and composed, easily offended, cannot bear slightest opposition, feels being noticed by
others, fear of crowd, easily crying on grief, sad music, Fear of robbers.

On examination
General physical examination
The patient was of lean thin, with 5.2" height and 46 kg weight.
Body mass index (BMI) was 20.9.
Blood pressure was 110/80 mmHg
Pulse- 89/min
Respiration 16/min.
Pallor-Absent,
Cyanosis-Absent
Clubbing-Absent

Systemic examination
On Inspection-swelling absent, on palpation-the thyroid gland was mobile, firm and non-tender.
Diagnostic Assessment Thyroid profile investigation dated 14.06.2022 2014 TSH level to be 8.8910 mlU/ML, withT3: 0.69 ng/ML and T4: 7.92 µg%.

On basis of case taking and totality of symptoms

- Fear general phobia noise from
- Fear of thunderstorm
- Consolation aggravates
- Hair fall
- Easily Offended
- Irregular and delayed menses
- Hot patient
- Feels better in cold air
- Profuse perspiration
- Dry skin
- Desires spicy food
- General aggravation from heat

MATERIALS AND METHODS

Case processing

Analaysis of symptoms

• Fear of noice • Fear of Thunderstrom • Consolation Aggravates • Hair fall • Desire for spicy food. • Easily offended • Delayed and irregular Menses •Hot Patient• Feels better in open Air• Profuse Perspiration • Dry skin•general aggravation from heat and sun exposure.

Mentals- fear of noice, Thunderstrom, consolation Aggravates, offended Easily, Physical Generals- Haifalls, Desire for spicy food, general aggravation from heat, better in cold air.

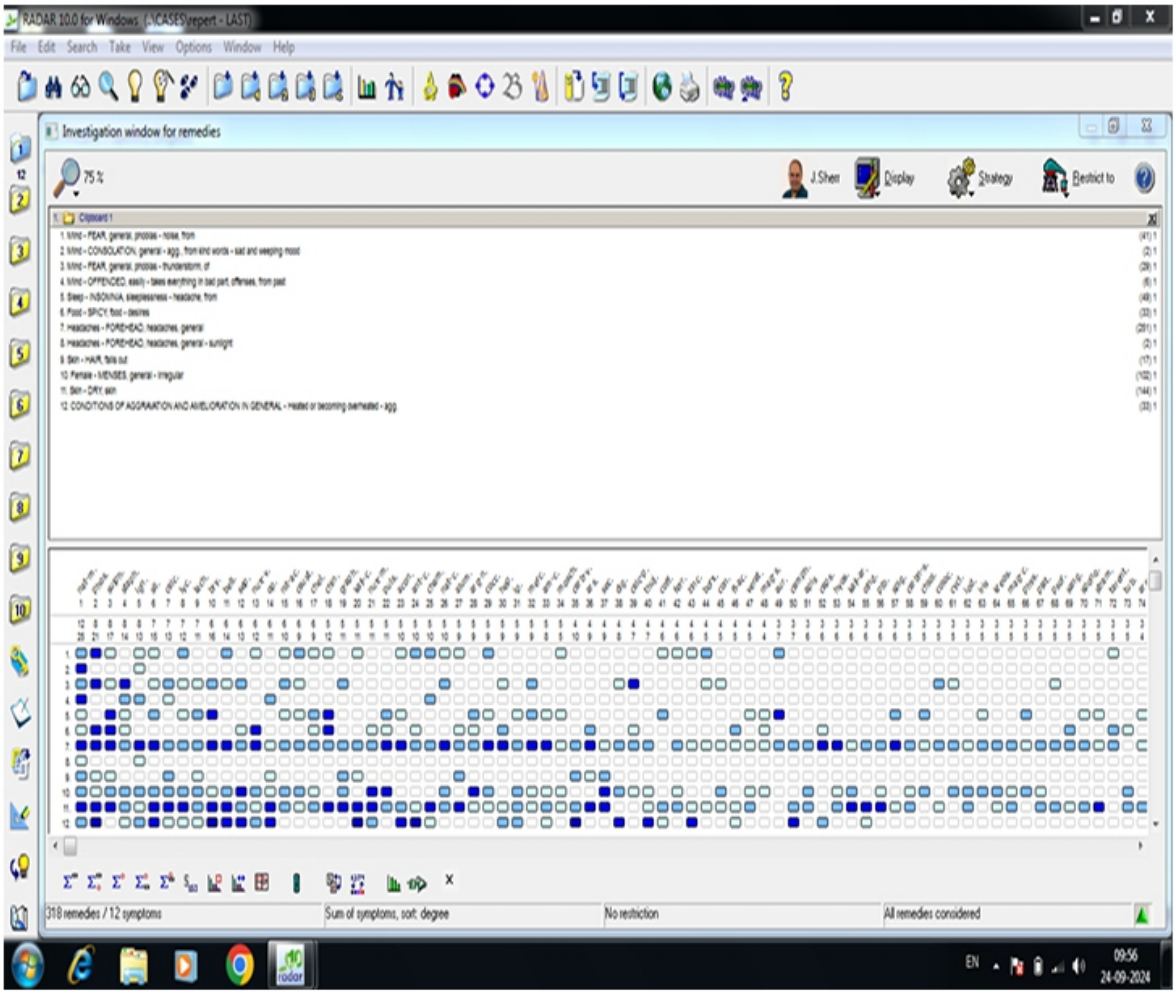
Particulars

Dryskin, profuse persipiration,

Totality of symtoms

- Fear general phobia noise from
- Fear of thunderstorm
- Consolation aggravates
- Hair fall
- Easily Offended
- Irregular and delayed menses
- Hot patient
- Feels better in cold air
- Profuse perspiration
- Dry skin
- Desires spicy food
- General aggravation from heat

Repertorisation After analysis and evaluation of the case, the following rubrics were taken for Repertorisation **(Figure 1).**



The Most Important and Major Polycrest Remedy from Mineral Kingdom Natrum Muraticum was chosen. For 15 days, the patient was instructed to take Natrum Muraticum 0/1 twice daily. To attain the results, the patient was followed-up every two weeks (Table 1). Discussion in this case, the patient presented with complaints of menstrual irregularities and hair fall which are common symptoms of hypothyroidism [6]. However, weakness during menses, hypothyroidism usually presents with intolerance to cold, loss of appetite and weight gain; whereas in this case, there was amelioration from cold air, and no weight gain (BMI: 20.9), which were peculiar. Medicine prescribed on the basis of peculiar and characteristic symptoms led to not only relief in signs and symptoms but also restoration of TSH levels.

Table 1. Follow-up and outcomes

Date of Follow-up	Symptoms and justification of prescription	Medicine with doses, Repetition
14 JUNE 2022	As reported on **14.06.2022** **TSH: 8.8910 uIU/mL** hair falls out, headache from lack of sleep, irregular and delayed menses	***Natrum Muraticum 0/1*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days

30 JUNE 2022	Sleep improved Hair fall reduced LMP was on **22.06.2022** Relief in weakness during menses.	***Natrum Muraticum 0/2*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days
16 JULY 2022	Hair fall: improved, Sleep-much better General Complains improved Burning better during Urination	***Natrum Muraticum 0/3*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days
2 AUGUST 2022	LMP was on **25.07.2022** Menses became regular. GC: Better Weakness during menses was much better. Hair fall: Better Mentally Sense of well being reported	***Natrum Muraticum 0/4*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days
17 AUGUST 2022	Hair loss greatly decreased All symptoms were better.	***Natrum Muraticum 0/5*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days
31 AUGUST 2022	LMP was on **24.08.2022** Hair loss stopped almost 90% as per Patient's version, Remarkable Improvement in sleep pattern. General complaints improved Hair loss stopped almost 90% as per patient reported	***Natrum Muraticum 0/6*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days
22 SEPTEMBER 2022	**TSH is 3.6841 mlU/mL** as reported on **22 September 2022** Menses became regular. GC: Better Weakness during menses was much better. Hair fall: Better Mentally Sense of well being reported	***Natrum Muraticum 0/7*** Give Ten uniform downward strokes 1 spoonful medicine to be taken and mixed with 1 cup of water To be mixed well and 1 spoonful mixed medicine to be taken in empty stomach BD/15 days

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MC-2091

LABORATORY REPORT

Name : [redacted]		Sex/Age : Female / 24 Years	BirthDay :
Case ID : 2061202615		Ref Id1 :	Mobile : 8320097552
Sample Type : EDTA Sample			Passport :
Reg Date and Time	: 14-Jun-2022 01:52 PM	Ref. By	: Dr. Parag Shah D.M.,DNB(Endocrinology)
Sample Date and Time	: 14-Jun-2022 01:52 PM	Pt. Loc	:
Report Date and Time	: 14-Jun-2022 03:40 PM	Bill. Loc.	:

Parameter	Results	Unit	Biological Ref. Interval
HAEMOGRAM			
Method : Electrical Impedence/Optical Analysis by Automated cell Counter			
Haemoglobin (Photometric)	13.20	gm%	12-15
RBC Count	4.53	mill/cmm	3.8-4.8
WBC Count	5900	/cmm	4000-10000
Platelet Count	213000	/cmm	150000-410000
DIFFERENTIAL WBC COUNT	[%]	EXPECTED VALUES	[Abs] EXPECTED VALUES
Polymorphs	54 %	40 - 80	3186 2000-7000
Lymphocyte	33	20 - 40	1947 1000-3000
Eosinophils	04	1-6%	236 20-500
Monocytes	09	2-10	531 200-1000
Basophils	00	<1-2	L 0 20-100
Blood Indices (Calculated)			
Hematocrit/PCV	39.00	%	36-46
MCV (Measured)	86.00	fL	83-101
MCH	29.10	Pg	27-32
MCHC	33.90	g/dL	31.5-34.5
RDW	13.80	cv%	11.6 - 14.0
MPV	9.10	fL	7.2 - 11.7
WBC MORPHOLOGY	Premature cells are not seen		
RBC MORPHOLOGY	Normocytic,Normochromic		
PLATELET MORPHOLOGY	Platelets are adequate on smear.		
Malarial Parasite	Malarial parasite not seen in smear		

Dr. Mansi Thakker MD(Path)
Dr. Amarjeet Kaur MD (Path.)

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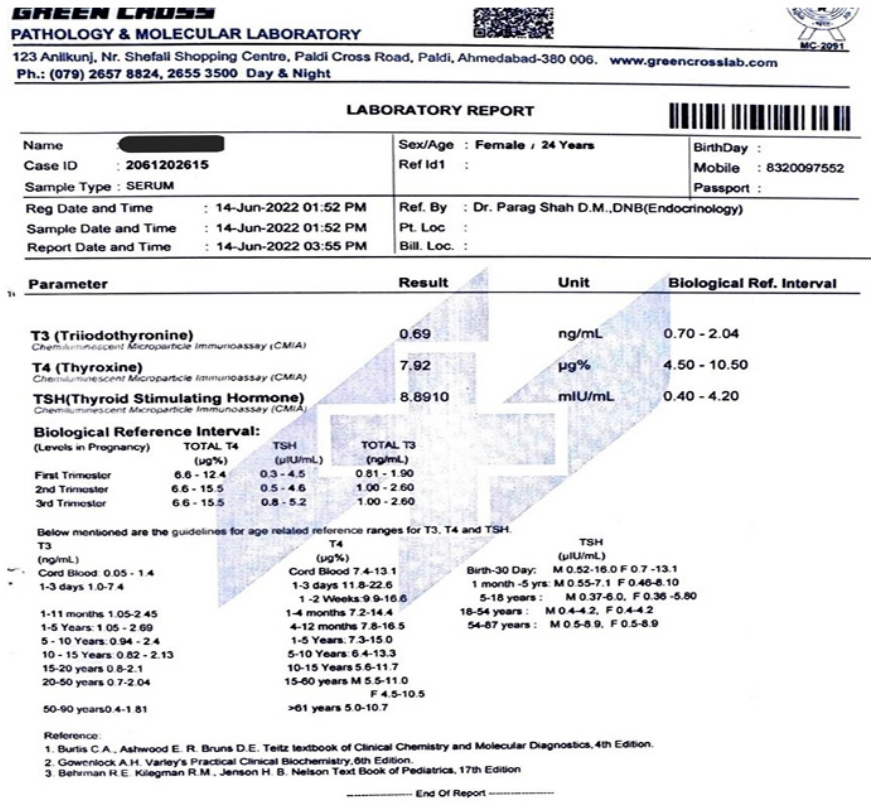
Figure 2. Investigation Reports: As on- 14.06.2022



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MC-2091

LABORATORY REPORT

Name : [redacted]		Sex/Age : Female / 24 Years	BirthDay :
Case ID : 2061202615		Ref Id1 :	Mobile : 8320097552
Sample Type : SERUM			Passport :
Reg Date and Time	: 14-Jun-2022 01:52 PM	Ref. By	: Dr. Parag Shah D.M.,DNB(Endocrinology)
Sample Date and Time	: 14-Jun-2022 01:52 PM	Pt. Loc	:
Report Date and Time	: 14-Jun-2022 03:55 PM	Bill. Loc.	:

Parameter	Result	Unit	Biological Ref. Interval
T3 (Triiodothyronine) *Chemiluminescent Microparticle Immunoassay (CMIA)*	0.69	ng/mL	0.70 - 2.04
T4 (Thyroxine) *Chemiluminescent Microparticle Immunoassay (CMIA)*	7.92	µg%	4.50 - 10.50
TSH(Thyroid Stimulating Hormone) *Chemiluminescent Microparticle Immunoassay (CMIA)*	8.8910	mIU/mL	0.40 - 4.20

Biological Reference Interval:

(Levels in Pregnancy)	TOTAL T4 (µg%)	TSH (µIU/mL)	TOTAL T3 (ng/mL)
First Trimester	6.6 - 12.4	0.3 - 4.5	0.81 - 1.90
2nd Trimester	6.6 - 15.5	0.5 - 4.6	1.00 - 2.60
3rd Trimester	6.6 - 15.5	0.8 - 5.2	1.00 - 2.60

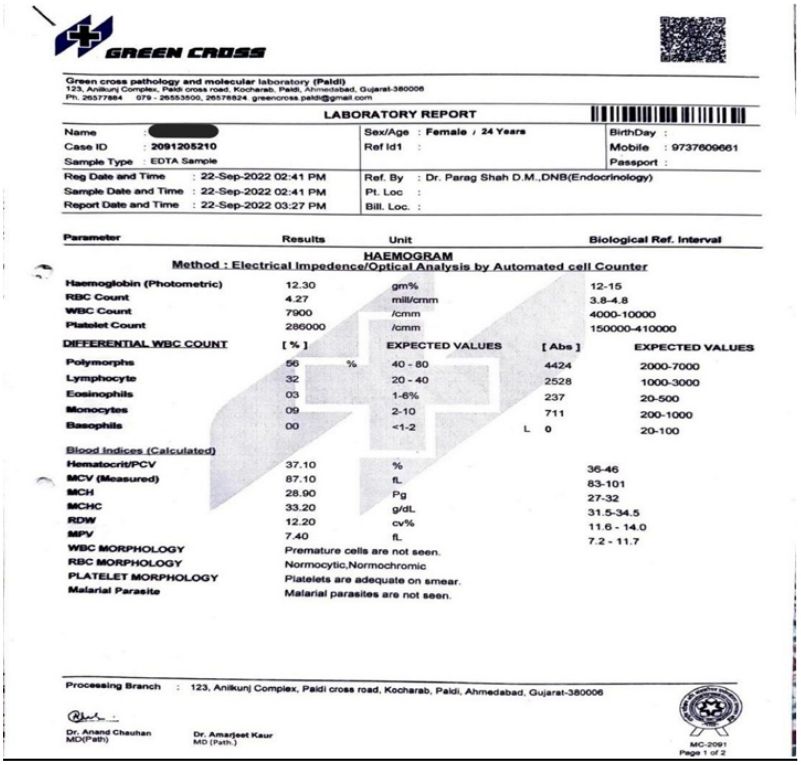
Below mentioned are the guidelines for age related reference ranges for T3, T4 and TSH.

T3 (ng/mL)	T4 (µg%)	TSH (µIU/mL)
Cord Blood 0.05 - 1.4	Cord Blood 7.4-13.1	Birth-30 Day: M 0.52-16.0 F 0.7 -13.1
1-3 days 1.0-7.4	1-3 days 11.8-22.6	1 month -5 yrs: M 0.55-7.1 F 0.46-8.10
	1 -2 Weeks:9.9-16.6	5-18 years : M 0.37-6.0, F 0.36 -5.80
1-11 months 1.05-2.45	1-4 months 7.2-14.4	18-54 years : M 0.4-4.2, F 0.4-4.2
1-5 Years: 1.05 - 2.69	4-12 months 7.8-16.5	54-87 years : M 0.5-8.9, F 0.5-8.9
5 - 10 Years: 0.94 - 2.4	1-5 Years: 7.3-15.0	
10 - 15 Years: 0.82 - 2.13	5-10 Years: 6.4-13.3	
15-20 years 0.8-2.1	10-15 Years 5.6-11.7	
20-50 years 0.7-2.04	15-60 years M 5.5-11.0 F 4.5-10.5	
50-90 years0.4-1.81	>61 years 5.0-10.7	

Reference:
1. Burtis C.A., Ashwood E. R. Bruns D.E. Teitz textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition.
2. Gowenlock A.H. Varley's Practical Clinical Biochemistry, 6th Edition.
3. Behrman R.E. Kliegman R.M., Jenson H. B. Nelson Text Book of Pediatrics, 17th Edition

---------- End Of Report ----------

Figure 3. Thyroid Profile As on 14.06.2022



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LABORATORY REPORT

Name	[redacted]	Sex/Age : Female / 24 Years	BirthDay :
Case ID	: 2091205210	Ref Id1 :	Mobile : 9737609661
Sample Type	: EDTA Sample		Passport :
Reg Date and Time	: 22-Sep-2022 02:41 PM	Ref. By	: Dr. Parag Shah D.M.,DNB(Endocrinology)
Sample Date and Time	: 22-Sep-2022 02:41 PM	Pt. Loc	:
Report Date and Time	: 22-Sep-2022 03:27 PM	Bill. Loc.	:

HAEMOGRAM

Method : Electrical Impedence/Optical Analysis by Automated cell Counter

Parameter	Results	Unit	Biological Ref. Interval
Haemoglobin (Photometric)	12.30	gm%	12-15
RBC Count	4.27	mill/cmm	3.8-4.8
WBC Count	7900	/cmm	4000-10000
Platelet Count	286000	/cmm	150000-410000

DIFFERENTIAL WBC COUNT	[%]	EXPECTED VALUES	[Abs]	EXPECTED VALUES
Polymorphs	56 %	40 - 80	4424	2000-7000
Lymphocyte	32	20 - 40	2528	1000-3000
Eosinophils	03	1-6%	237	20-500
Monocytes	09	2-10	711	200-1000
Basophils	00	<1-2	L 0	20-100

Blood Indices (Calculated)			
Hematocrit/PCV	37.10	%	36-46
MCV (Measured)	87.10	fL	83-101
MCH	28.90	Pg	27-32
MCHC	33.20	g/dL	31.5-34.5
RDW	12.20	cv%	11.6 - 14.0
MPV	7.40	fL	7.2 - 11.7
WBC MORPHOLOGY	Premature cells are not seen.		
RBC MORPHOLOGY	Normocytic,Normochromic		
PLATELET MORPHOLOGY	Platelets are adequate on smear.		
Malarial Parasite	Malarial parasites are not seen.		

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Figure 4. Investigation Report as on 22.09. 2022

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LABORATORY REPORT

Name	[redacted]	Sex/Age : Female / 24 Years	BirthDay :
Case ID	: 2091205210	Ref Id1 :	Mobile : 9737609661
Sample Type	: SERUM		Passport :
Reg Date and Time	: 22-Sep-2022 02:41 PM	Ref. By	: Dr. Parag Shah D.M.,DNB(Endocrinology)
Sample Date and Time	: 22-Sep-2022 02:41 PM	Pt. Loc	:
Report Date and Time	: 22-Sep-2022 04:26 PM	Bill. Loc.	:

Parameter	Result	Unit	Biological Ref. Interval
T3 (Triiodothyronine) (Chemiluminescent Microparticle Immunoassay (CMIA))	0.79	ng/mL	0.70 - 2.04
T4 (Thyroxine) (Chemiluminescent Microparticle Immunoassay (CMIA))	8.44	µg%	4.50 - 10.50
TSH(Thyroid Stimulating Hormone) (Chemiluminescent Microparticle Immunoassay (CMIA))	3.6841	mIU/mL	0.40 - 4.20

Biological Reference Interval:

(Levels in Pregnancy)	TOTAL T4 (µg%)	TSH (µIU/mL)	TOTAL T3 (ng/mL)
First Trimester	6.6 - 12.4	0.3 - 4.5	0.81 - 1.90
2nd Trimester	6.6 - 15.5	0.5 - 4.6	1.00 - 2.60
3rd Trimester	6.6 - 15.5	0.8 - 5.2	1.00 - 2.60

Below mentioned are the guidelines for age related reference ranges for T3, T4 and TSH.

T3 (ng/mL)	T4 (µg%)	TSH (µIU/mL)
Cord Blood 0.05 - 1.4	Cord Blood 7.4-13.1	Birth-30 Day: M 0.52-16.0 F 0.7 -13.1
1-3 days 1.0-7.4	1-3 days 11.8-22.6	1 month -5 yrs: M 0.55-7.1 F 0.45-9.19
1-11 months 1.05-2.45	1 -2 Weeks 9.9-16.6	5-18 years M 0.37-6.6, F 0.36 -5.80
1-5 Years: 1.05 - 2.65	1-4 months 7.2-14.4	18-54 years : M 0.4-4.2, F 0.4-4.2
5 - 10 Years: 0.94 - 2.4	4-12 months 7.8-16.5	54-87 years : M 0.5-8.9, F 0.5-8.9
10 - 15 Years: 0.82 - 2.13	1-5 Years: 7.3-15.0	
15-20 years 0.8-2.1	5-10 Years: 6.4-13.3	
20-50 years 0.7-2.04	10-15 Years 5.5-11.7	
50-90 years0.4-1.81	15-60 years M 5.5-11.0 F 4.5-10.5	
	>61 years 5.0-10.7	

Reference:
1. Burtis C.A., Ashwood E. R. Bruns D.E. Tietz textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition.
2. Glowniock A.H. Varley's Practical Clinical Biochemistry, 6th Edition.
3. Behrman R.E. Kliegman R.M., Jenson H. B. Nelson Text Book of Pediatrics, 17th Edition

End Of Report

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Dr. Anand Chauhan Dr. Amarjeet Kaur

Figure 5. Thyroid profile As on 22.09.2022

RESULTS AND DISCUSSION

In Homoeopathy we treat the patient (individual) not the disease by merely its name and diagnosis. Our approach to every individual case is to improve the vitality and immunity of the patient with our medicines so that the patient can get back to normalcy without any adverse and unwanted complications. Whereas same may not be said for the conventional system of medicine. This case presented with elevated level of TSH and the patient was already under medication for the same for almost a year. On her first visit (14.07.2022) the lady presented with elevated TSH level which was 8.8910mIU/mL (Figure 3). Natrum Muraticum 0/1 was the first prescription on basis of Totality of Symptoms and reportorial Report and subsequent consultation with Materia Medica.and on her subsequent visits the potency was increased and her TSH Level came down to 3.684 mIU/Ml (Figure 5) which is with in normal limits with disappearance of symptoms of fear, Hair fall, menstrual irregularities. A normal range of TSH level in a short time span of 4 months with non-recurrence of complaints is documentary evidence in favour of homoeopathy. Various research studies have shown efficacy of homeopathy in successful treatment of sub-clinical Hypothyroidism [7].

CONCLUSION

This case definitely confirms the Efficacy of homoeopathy for managing such endocrine conditions. as it is clearly evident that particularly with this patient even after taking Thyronorm for almost a year she was not experiencing any relief from her complaints. Rather her condition was becoming worse, which prompted her doctor to advice an increased dose of medicine. whereas on proper and detailed history taking based on Dr Hahnemann's homoeopathic Principles we could provide her relief from not only physical Symptoms but also her mental Anxiety, Her Also fear improved.

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REFERENCES

1. Harsh M. Text book of pathology. 9th ed. Jaypee Brothers medical Publishers (P) Ltd; 2023. p. 816-7.
2. Longo DL, Fauci AS, Kasper DL, Hauser SL. Harrison's principles of internal medicine. 21st ed. McGraw Hill Education; 2022.
3. Architch B, Ramdas N. Exam preparatory manual for undergraduates –medicine. 4th ed. Jaypee Brothers; 2023. p. 1010-1.
4. Harsh M. Text book of pathology. 9th ed. Jaypee Brothers medical Publishers (P) Ltd; 2023. p. 843-4.
5. Davidson's Principles & Practice of Medicines. 24th ed. Churchill Livingstone ELSEVIER; 2023. p. 657.
6. Murphy's Repertory, RADAR 10 Homoeopathic software.

7. Chauhan VK, Manchanda RK, Narang A, Marwaha RK, Arora S, Nagpal L, et al. Efficacy of homeopathic intervention in subclinical hypothyroidism with or without autoimmune thyroiditis in children: an exploratory randomized control study. Homeopathy. 2014;103(04):224-31.

Modern Pharmacological Treatment of Parkinson's Disease: Reviving Known Drugs and New Perspectives

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ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor symptoms such as tremor and rigidity, along with non-motor symptoms such as cognitive decline and depression. Current dopaminergic therapies offer symptomatic relief but fail to halt disease progression, underscoring the urgent need for novel, disease-modifying therapies. This review explores the potential of repurposed drugs from different therapeutic categories—including immunomodulatory, cardiometabolic, and anti-infective agents—as promising therapeutic strategies for PD. Immunomodulatory agents such as c-Abl inhibitors (imatinib, nilotinib) and sargramostim have shown potential in reducing α-synuclein aggregation and neuroinflammation, although clinical outcomes have been mixed. Cardiometabolic drugs, particularly glucagon-like peptide-1 agonists like exenatide, have shown improvements in motor and cognitive symptoms, with ongoing phase III trials evaluating their disease-modifying potential. Anti-infective agents, including doxycycline and rifampicin, exhibit neuroprotective effects through anti-inflammatory and anti-aggregating effects. While some concerns regarding efficacy and toxicity persist, these repurposed drugs offer valuable insights into novel therapeutic approaches for PD. In addition, emerging strategies such as gene therapy, enzyme replacement, and advanced drug delivery systems are discussed for their potential to address underlying disease mechanisms. Despite the lack of definitive disease-modifying therapies to date, advances in drug repurposing and innovative therapeutic approaches provide hope for future breakthroughs. Further large-scale clinical trials are necessary to confirm the efficacy and safety of these treatments.

Keywords: Parkinson's disease, Neurodegeneration, Drug repurposing, Immunomodulatory therapies, Disease modifying treatments

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily resulting from the loss of dopaminergic neurons in the substantia nigra (SN). These neurons play a crucial role in motor control, coordination, and various cognitive functions by producing dopamine. The depletion of dopamine leads to the hallmark motor symptoms (MS) of PD, which include tremors, increased muscle tone (rigidity), bradykinesia (slowness of movement), and postural instability, contributing to difficulties in gait and balance. In addition, PD progression varies significantly between individuals, and while the onset is often gradual, the disease inevitably worsens over time. Despite ongoing research, there is no disease-modifying treatment available for PD. However, symptomatic therapies and various interventions can alleviate MS and enhance the quality of life (QoL) for patients [1, 2]. In addition to the well-known MS, PD is also associated with a broad spectrum of non-motor symptoms (NMS), which can be equally, if not more, debilitating for patients. These NMS include dysarthria (speech difficulties), dysphagia (swallowing difficulties), depression, anxiety, sleep disturbances, and cognitive impairments such as memory loss and executive dysfunction. Other prevalent NMS include autonomic dysfunctions such as

constipation and seborrheic skin disorders [3-6]. The severity and manifestation of these symptoms vary widely among patients, with not all experiencing the full spectrum of NMS. These deficits have a substantial impact on patient outcomes and make clinical management of PD more difficult. PD affects approximately 1 million individuals in the United States alone, with an average age of onset around 60 years [7]. The annual incidence rate in the U.S. is estimated at 60,000 new cases [8]. Moreover, the prevalence increases with age, affecting 1-2% of individuals over 60 years old, and rising to nearly 4% in individuals over 80 years old [9]. The disease is more prevalent in men than in women, with a male-to-female ratio of about 1.5 to 1 [10]. Additionally, PD is more common among white populations compared to other racial or ethnic groups [11]. Globally, the burden of PD is expected to rise as the population ages, making it a significant public health challenge [12, 13]. Given the current limitations in treating PD and the anticipated rise in its global prevalence, there is an urgent need to explore new therapeutic fields. Recent research has started to investigate the potential repurposing of drugs from other medical fields, such as cardiology and immunology, for PD treatment. This approach involves targeting new molecular pathways and mechanisms that may help alleviate symptoms or slow disease progression. In this review, we have synthesized the latest findings in this area, particularly focusing on repurposed medications such as immunosuppressants and cardiovascular drugs. Our objective is to enhance understanding of these emerging therapies and discuss their potential to open new frontiers in PD management, aiming to improve patient outcomes and QoL.

RESULTS AND DISCUSSION

Background and disease pathology

At the molecular level, PD is thought to be related to a combination of abnormal protein accumulation, inflammation, and the generation of reactive oxygen species (ROS), all of which contribute to the degeneration of neurons in the SN (Figure 1). A hallmark of PD is the formation of abnormal protein aggregates known as Lewy bodies (LBs) within the nerve cells of the SN, which are primarily composed of alpha-synuclein, a protein expressed throughout the brain [14]. These aberrant protein aggregates cause neurons' regular functions to be disrupted, which eventually results in their death. Beyond PD, LBs are also a feature of other neurodegenerative illnesses, like multiple system atrophy (MSA) and dementia with LBs, where their existence is associated with both motor and cognitive symptoms [15]. The role of alpha-synuclein in the development of LBs has been extensively studied. Alpha-synuclein is a 140 kDa protein encoded by the SNCA gene, and it is suggested to regulate neurotransmitter release and maintain the presynaptic cytoskeleton, as well as facilitate vesicular transport within cells [16-18]. Although its exact function remains unclear, alpha-synuclein is believed to play a critical role in maintaining neuronal integrity and modulating ion channel activity [18]. Furthermore, studies suggest that alpha-synuclein is involved in the regulation of oxidative stress and apoptosis [19, 20]. Research indicates that alpha-synuclein may promote apoptosis through interactions with mitochondria, which are essential for both energy production and the regulation of cell death [21, 22]. Genetic factors, including mutations in the SNCA gene and other related genes, have been implicated in the formation of LBs and the development of familial PD, though this form of the disease is rare [23, 24]. These genetic mutations are thought to increase susceptibility to PD and other alphasynucleinopathies, highlighting the potential influence of heritable factors in what has traditionally been considered a sporadic or idiopathic disease [24].

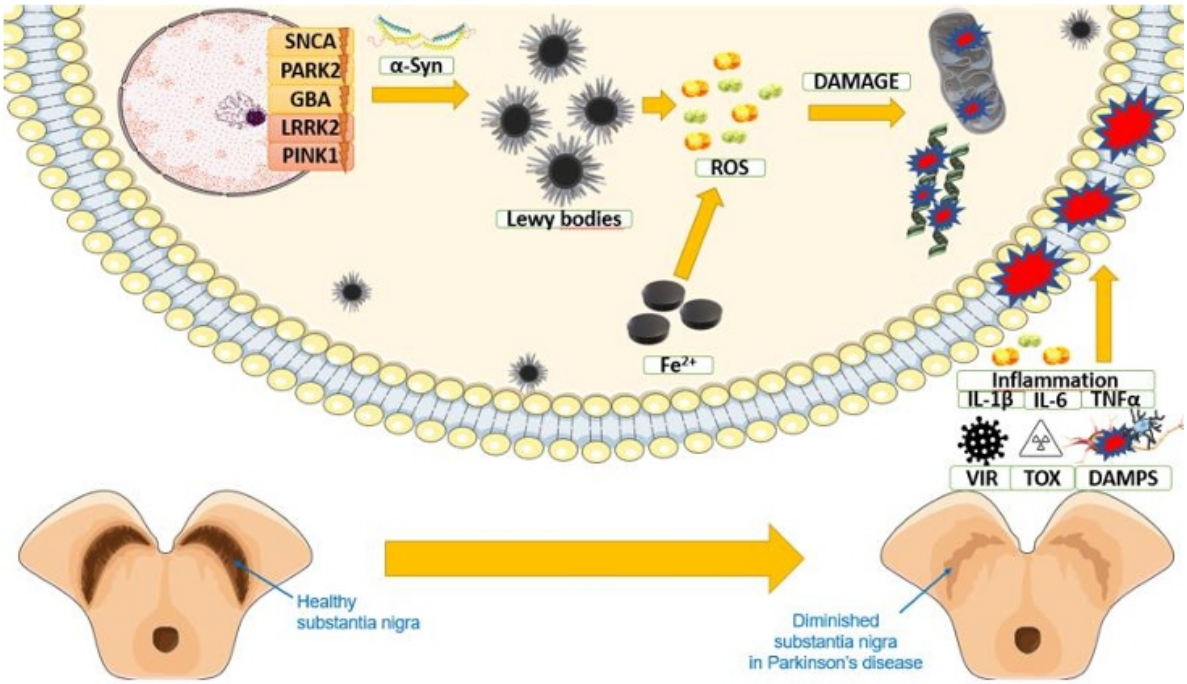

Figure 1. The pathophysiology model of PD. Abbreviations: SNCA, Synuclein Alpha Gene; PARK2, Parkin Gene; GBA, Glucocerebrosidase Gene; LRRK2, Leucine-Rich Repeat Kinase 2 Gene; PINK1, PTENinduced kinase 1; α-Syn, Alpha-Synuclein; ROS, Reactive Oxygen Species; Fe^{2+}, Iron ions (Ferrous); IL-1β, Interleukin-1 Beta; IL-6, Interleukin-6; TNFα, Tumor Necrosis Factor Alpha; VIR, Viruses; TOX, Toxins; and DAMPS, Damage-Associated Molecular Patterns.

LBs are not exclusive to the SN; they can also be found in other regions of the brain, such as the amygdala, hippocampus, hypothalamus, and brainstem nuclei like the locus coeruleus, nucleus basalis of Meynert, and dorsal motor nucleus of the vagus nerve. They may also appear in the neocortex and amygdala in some cases of PD. Research by Braak and Tredici further revealed that LBs can extend beyond the central nervous system (CNS) to peripheral and enteric nervous systems, suggesting a broader involvement of these aggregates in PD pathology. In addition to alpha-synuclein, LBs are composed of other proteins such as tau, ubiquitin, and neurofilaments, which may contribute to their formation and stability [25]. Other proteins, including 14-3-3, DJ-1, parkin, and LRRK2, have also been detected in LBs, though their precise roles remain unclear and warrant further investigation [25, 26]. Understanding the function of these proteins within LBs, as well as their contributions to the pathogenesis of neurodegenerative disorders like PD, is critical for developing potential therapeutic interventions targeting this molecular pathology.

Repurposed drugs for PD treatment- results

• *Immunomodulatory and anti-neurodegenerative therapies*

Abelson tyrosine kinase inhibitors

Abelson Tyrosine Kinase (c-Abl): Abelson tyrosine kinase (c-Abl) is a non-receptor tyrosine kinase crucial for cellular stress response. Studies on animal models of PD suggest that the activation of c-Abl

contributes to the accumulation of α-Synuclein (α-Syn) and neuronal degradation, making c-Abl a potential target for diseasemodifying therapies [27]. Imatinib, the first identified c-Abl inhibitor, has been used successfully in the treatment of Chronic Myeloid Leukemia (CML) and gastrointestinal stromal tumors. Research in MPTP-induced mouse models of PD demonstrated the neuroprotective effects of Imatinib. Administering 30 mg/kg of Imatinib significantly reduced c-Abl tyrosine phosphorylation and protected dopaminergic neurons from degeneration [28]. Nilotinib is a second-generation c-Abl inhibitor with higher selectivity and better brain penetration compared to other inhibitors. Several clinical trials have assessed its efficacy in PD patients. In a phase 2, double-blind, placebo-controlled study with 63 participants, nilotinib was shown to be safe with no significant adverse effects. However, no meaningful clinical improvements in MS were observed [29, 30]. Another study confirmed nilotinib's safety but did not show significant motor or non-motor outcome improvements compared to the placebo group [31]. Nevertheless, nilotinib has been shown to alter dopamine metabolism by increasing the levels of DOPAC and homovanillic acid (HVA), indicating potential neurochemical effects that warrant further research [31].

Sargramostim

Sargramostim is a recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) that stimulates immune function by activating neutrophils, macrophages, and myeloid dendritic cells. Given the involvement of mitochondrial and peripheral immune dysfunction in PD, sargramostim has emerged as a potential long-term therapeutic option [32, 33]. Sargramostim was well tolerated in PD patients, according to a phase 1 randomized, placebo-controlled research. Adverse events included injection site reactions and mild bone discomfort [34]. In addition, sargramostim increased regulatory T-cell (Treg) subsets and improved MDS-UPDRS Part III scores after six to eight weeks of treatment. A subsequent phase 1b study with reduced sargramostim dosing (3 mg/kg/day) showed fewer adverse events without worsening MS, though larger studies are necessary to determine its therapeutic efficacy [35].

Rapamycin (Sirolimus)

Rapamycin, an mTOR (mammalian target of rapamycin) inhibitor, has been used as an immunosuppressant to prevent organ transplant rejection. In PD models, mTOR inhibition by rapamycin has been shown to activate autophagy, which helps clear α-Syn aggregates, reduce oxidative stress, and alleviate dopaminergic neuronal damage [36]. Studies on mice with parkin and PINK1 mutations indicate that rapamycin improves PD-related pathology by reducing muscle and mitochondrial degeneration [37]. However, more human trials are required to validate these results and assess rapamycin's therapeutic potential in individuals with PD.

Isoalantolactone

Isoalantolactone is a bioactive sesquiterpene lactone known for its anti-inflammatory and anti-tumor properties. Research has shown that IAL can prevent Amyloid β-induced toxicity and ameliorate MPTP-induced PD symptoms in mouse models [38, 39]. The neuroprotective effects of IAL are thought to be mediated through its activation of antioxidant pathways, specifically by stimulating the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway [40]. IAL treatment has also been shown to preserve dopaminergic neurons and reduce neuroinflammation, making it a promising candidate for PD treatment. However, further research is needed to elucidate its molecular mechanisms and therapeutic potential.

Interferon beta IFN-β is a polypeptide drug widely used in the treatment of relapsing multiple sclerosis (MS). Recent studies indicate that IFN-β can reduce neuroinflammation and prevent neurodegeneration in PD models. IFN-β has been shown to promote α-Syn degradation and protect dopaminergic neurons by modulating mitochondrial dynamics through the STAT5-PGAM5-Drp1 pathway [41, 42]. In vivo models of PD have demonstrated that IFN-β prevents neuronal loss and oxidative damage. However, more research is needed to fully understand the neuroprotective mechanisms of IFN-β and its potential for PD therapy.

• *Cardiological and metabolic (anti-diabetic) drugs*

Glucagon-like peptide 1 (GLP-1) agonists

Exenatide, a GLP-1 agonist, has emerged as one of the most studied drugs for potential repurposing in PD treatment. An open-label study showed a clinically significant improvement in both motor and cognitive symptoms in patients with moderate PD, with a mean improvement of 2.7 in the MDS-UPDRS scale compared to a decline of 2.2 in the control group (P = 0.037). Weight loss was the only notable adverse effect [43, 44]. Exenatide also showed the potential to slow down disease progression, as demonstrated in another clinical trial, where participants exhibited a mean improvement of 1.0 in MDS-UPDRS after 60 weeks of treatment (95% CI = -2.6 to 0.7) [45, 46]. Exenatide is currently completing phase III trials, and more research is being done on its neuroprotective, anti-apoptotic, anti-inflammatory, and antioxidative qualities [47]. Other GLP-1 agonists, including liraglutide and lixisenatide, are in phase II trials [48]. Semaglutide has shown potential by improving motor dysfunction, reducing α-Syn aggregation, and increasing glial cell line-derived neurotrophic factor (GDNF) expression in PD mouse models [43].

The relationship between type 2 diabetes mellitus (T2DM) and PD has been recognized since the 1960s. A meta analysis of seven population-based cohort studies reported a 38% increased risk of developing PD among diabetic patients [49, 50]. Studies have shown widespread insulin resistance and impaired insulin signaling in the brains of PD patients [47]. Observational studies indicate that PD patients have lower fasting plasma insulin levels and a higher fasting plasma amylin/fasting plasma insulin ratio (FPAIR), with FPAIR showing a modest correlation with NMS (NMSS scale) [51]. Insulin has neuroprotective effects through the PI3K pathway, which may protect dopaminergic neurons from the harmful effects of hyperglycemia [52]. Research on intranasal insulin in rats has demonstrated encouraging outcomes in terms of promoting neurogenesis and warding off inflammation and ROS [53]. Nevertheless, intranasal insulin did not show appreciable functional or cognitive advantages in phase II/III trials for Alzheimer's disease and moderate cognitive impairment [54].

Statins

Among statins, simvastatin has garnered the most attention due to its superior blood-brain barrier permeability compared to other statins like pravastatin and rosuvastatin. Preclinical studies in PD mouse models (6-OHDA and MPTP-induced) demonstrated simvastatin's neuroprotective effects [55]. Although cohort studies suggested that simvastatin may reduce PD risk, other retrospective case-control studies provided conflicting results [56]. A randomized controlled trial (RCT) in the UK enrolled 235 patients to assess simvastatin’s disease-modifying potential in PD. Despite the trial’s rigorous design, the results did not support simvastatin as a disease-modifying agent, and further trials were not pursued [57].

Metformin

Metformin, a widely used antidiabetic drug, has shown promising neuroprotective effects in vitro and in vivo. These include the inhibition of α-Syn phosphorylation and aggregation, the alleviation of oxidative stress, the prevention of mitochondrial dysfunction, the modulation of autophagy through the AMPK pathway, and the inhibition of glial cell hyperactivation [58, 59]. Despite these promising preclinical findings, recent meta-analyses suggest that metformin use in humans does not correlate with reduced PD risk and may even increase the risk, particularly in monotherapy [60]. Issues with metformin's bioavailability in the brain and potential adverse effects from long-term use may explain these contradictions. New stratification criteria for identifying PD patients who could benefit from metformin are being explored, including studies on patients with idiopathic REM sleep behavior disorder, a group at high risk for PD [61, 62].

Sodium-glucose cotransporter-2 (SGLT2) inhibitors

The pathogenesis of both PD and T2DM shares mechanisms such as mitochondrial dysfunction and oxidative stress. SGLT2 inhibitors (flozins) may offer neuroprotective effects through their glucose excretion properties, lowering glycated hemoglobin, and reducing ROS production by inhibiting NADPH oxidase activity. These actions protect mitochondrial function and reduce inflammation [63]. In a murine model of PD, dapagliflozin improved motor dysfunction reduced oxidative stress, and attenuated ROS-dependent apoptosis [64]. A large population-based study comparing SGLT2 inhibitors with dipeptidyl peptidase-4 inhibitors (DPP4i) found a lower risk of PD in the SGLT2 group (HR = 0.28; 95% CI: 0.09 to 0.91; p = 0.0349) [65].

Deferiprone

Ferroptosis, an iron-dependent form of cell death, has been implicated in PD pathology, with increased iron accumulation observed in the brains of PD patients. Deferiprone, an iron chelator, has shown efficacy in reducing oxidative stress, improving motor activity, and preserving dopamine levels in preclinical PD models [55, 66]. Positive results from small-scale human studies also prompted the start of larger RCTs, such as the 372-participant FAIR PARK II experiment. Regretfully, further findings revealed that deferiprone was linked to worse parkinsonism scores among PD patients who had not yet started dopaminergic therapies, undermining the drug's potential as a therapeutic intervention [67, 68].

Antihypertensive drugs

Calcium channel blockers (CCBs)

Epidemiological studies suggest that CCBs, particularly isradipine, may reduce PD risk. However, the STEADYPD phase III RCT, involving 336 patients, failed to demonstrate isradipine's efficacy in slowing PD progression [56, 69]. While a modest impact on delaying the need for antiparkinsonian treatments was observed, the study highlighted dosage limitations as a potential issue [70]. Computational analysis using IBM Watson identified nifedipine as another potential candidate, though it has been associated with Parkinsonism syndromes in some reports [71, 72].

Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs)

Preclinical evidence supports the role of the renin-angiotensin system (RAS) in neuroinflammation and oxidative stress in PD, making ACE inhibitors and ARBs attractive therapeutic options. Studies have shown that drugs such as captopril and losartan can enhance L-DOPA effects without inducing dyskinesias and reduce PD risk in clinical populations [56, 73-77]. However, the quality of evidence

remains low, and further trials are necessary to validate these findings [72].

• *Anti-infective drugs in PD therapy*

Minocycline

Minocycline, a second-generation tetracycline antibiotic, exhibits efficacy against both gram-positive and gram negative bacteria. Owing to its lipophilicity, it can easily cross the blood-brain barrier (BBB) and accumulate in the cerebrospinal fluid (CSF) and CNS. This allows minocycline to be used in the treatment of CNS diseases, including PD. Minocycline exerts neuroprotective effects by inhibiting proinflammatory molecule production, reducing mitochondrial dysfunction, and preventing microglial activation, which are key processes in the etiopathogenesis of PD [78]. Preclinical studies in rotenone-induced PD rat models have demonstrated that minocycline can slightly alleviate motor deficits, although it has not shown significant improvements in motor function in clinical studies involving early PD patients [79].

Doxycycline

Doxycycline, another second-generation tetracycline antibiotic, is commonly used for bacterial infections. Recent studies highlight its ability to inhibit α-Syn aggregation and reduce mitochondrial-derived ROS [80]. In an experiment involving human α-Syn A53T transgenic mice, doxycycline treatment (10 mg/kg daily for 30 days) significantly improved motor function, including gait stability and muscle strength [81]. This suggests that doxycycline could be a potential therapeutic option for addressing both MS and neuropathological changes in PD. A randomized, double-blind, placebo-controlled trial is currently recruiting PD patients to further assess doxycycline's effects on motor performance and cognitive function in individuals receiving levodopa (NCT05492019).

Geldanamycin

Geldanamycin is an ansamycin antibiotic originally developed as an anticancer drug. It inhibits the function of Heat Shock Protein 90 (Hsp90) and upregulates Heat Shock Protein 70 (Hsp70). Hsp70 is known to prevent αSyn misfolding and reduce amyloid aggregation [82]. Inhibition of Hsp90 by geldanamycin has shown protective effects against MPTP-induced dopaminergic neurotoxicity in PD models, due to the induction of Hsp70, which counters neurotoxicity and mitochondrial stress [83]. Despite these promising results, the toxicity of geldanamycin limits its clinical application. However, structural analogs of geldanamycin, such as 17-AAG, 17-DMAG, IPI493, and retaspimycin, are currently being evaluated in clinical trials as potential treatments for PD [84].

Rifampicin

Rifampicin, an ansamycin antibiotic primarily used to treat mycobacterial infections, has demonstrated antiinflammatory, anti-aggregating, and antioxidant properties, making it a potential therapeutic candidate for neurological disorders like PD [84]. Rifampicin has been shown in experimental tests to lessen neuroinflammation and neurodegeneration brought on by fibrillary aggregates of α-Syn [85]. Rifampicin reduced apoptosis in zebrafish models of PD caused by rotenone through the reduction of pro-inflammatory cytokines such as IL-1β and IL-6 and the mitigation of mitochondrial oxidative stress [86]. These results suggest that rifampicin could modulate neuroinflammation and reduce mitochondrial dysfunction in PD, though further evaluation in clinical settings is needed.

Ceftriaxone

Ceftriaxone, a third-generation cephalosporin antibiotic, can cross the BBB and has shown neuroprotective effects in CNS disorders by upregulating excitatory amino acid transporter 2 (GLT-1) expression [87]. Chronic administration of ceftriaxone (200 mg/kg) in MPTP-induced PD rat models resulted in significant improvements in motor function and reduced oxidative damage. Furthermore, ceftriaxone downregulated neuroinflammation markers such as glial fibrillary acidic protein (GFAP) and Toll-like receptor 4 (TLR4) while reducing proinflammatory cytokines (IL-1β, TNF-α, and IL-6) [88]. A phase II randomized, double-blind, placebo-controlled trial is underway to assess the efficacy and safety of ceftriaxone in patients with PD dementia (NCT03413384).

Niclosamide

Niclosamide, an antihelminthic drug, has been recognized for its ability to modulate mitochondrial phosphorylation and influence various signaling pathways, including mTOR and JAK/STAT3 [89]. Niclosamide has shown promise in activating PINK1, a kinase involved in protecting against mitochondrial dysfunction, which is particularly relevant in autosomal recessive PD [90]. Niclosamide also promotes neurite growth in dopaminergic neurons and protects against α-Syn-induced neurodegeneration through the BMP-Smad pathway [91]. Although niclosamide shows potential as a neuroprotective agent, further research in vivo PD models is required to determine its effectiveness and safety.

CONCLUSION

This study highlights the potential of repurposed drugs for PD, focusing on immunomodulatory, cardiometabolic, and anti-infective agents. Immunomodulators like c-Abl inhibitors (imatinib, nilotinib) show neuroprotective effects by targeting α-Syn aggregation and cellular stress pathways, though clinical efficacy remains inconclusive. Cardiometabolic drugs, particularly GLP-1 agonists such as exenatide, demonstrate promising results in motor and cognitive symptom improvement, with potential disease-modifying effects. Anti-infective agents (minocycline, doxycycline, rifampicin) offer neuroprotection through anti-inflammatory and anti-aggregating actions, though clinical translation is limited by mixed results and toxicity concerns. While these drugs offer promising therapeutic strategies, further large-scale trials are necessary to confirm their efficacy and safety. A deeper understanding of their molecular mechanisms in PD could guide the development of optimized treatment approaches.

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FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Church FC. Treatment options for motor and non-motor symptoms of Parkinson's disease. Biomolecules. 2021;11(4):612.

2. DeMaagd G, Ashok P. Parkinson's disease and its management part 1: Disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. BMJ. 2015;308(6923):281.
3. Hermanowicz N, Jones SA, Hauser RA. Impact of non-motor symptoms in Parkinson's disease: A PMDAlliance survey. Neuropsychiatr Dis Treat. 2019;15:2205-12. doi:10.2147/NDT.S213917
4. Cosentino G, Avenali M, Schindler A, Pizzorni N, Montomoli C, Abbruzzese G, et al. A multinational consensus on dysphagia in Parkinson's disease: Screening, diagnosis and prognostic value. J Neurol. 2022;269(3):1335-52.
5. Mantovani S, Smith SS, Gordon R, O'Sullivan JD. An overview of sleep and circadian dysfunction in Parkinson's disease. J Sleep Res. 2018;27(3):1-22.
6. Tan AH, Lim SY, Chong KK, A Manap MAA, Hor JW, Lim JL, et al. Probiotics for constipation in Parkinson's disease: A randomized placebo-controlled study. Neurology. 2021;96(5):772-82.
7. National Institute of Neurological Disorders and Stroke. Parkinson's disease: Challenges, progress, and promise. 2023.
8. Marras C, Beck JC, Bower JH, Roberts E, Ritz B, Ross GW, et al. Prevalence of Parkinson's disease across North America. npj Park Dis. 2018;4(1):1-7.
9. Willis AW, Roberts E, Beck JC, Fiske B, Ross W, Savica R, et al. Incidence of Parkinson's disease in North America. npj Park Dis. 2022;8(1):1-12.
10. Rizek P, Kumar N, Mandar SJ. Diagnosis and treatment of Parkinson's. Acad Med Ont Southwest. 2016;188(16):1157-65.
11. Ray Dorsey E, Elbaz A, Nichols E, Abd-Allah F, Abdelalim A, Adsuar JC, et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: A systematic analysis for the global burden of disease study 2016. Lancet Neurol. 2018;17(11):939-53.
12. Gómez-Benito M, Granado N, García-Sanz P, Michel A, Dumoulin M, Moratalla R. Modeling Parkinson's disease with the alpha-synuclein protein. Front Pharmacol. 2020;11:1-15.
13. Ben-Joseph A, Marshall CR, Lees AJ, Noyce AJ. Ethnic variation in the manifestation of Parkinson's disease: A narrative review. J Parkinsons Dis. 2020;10(1):31-45.
14. Hu S, Tan J, Qin L, Lv L, Yan W, Zhang H, et al. Molecular chaperones and Parkinson's disease. Neurobiol Dis. 2021;160:105527.
15. Sulzer D, Edwards RH. The physiological role of α-synuclein and its relationship to Parkinson's Disease. J Neurochem. 2019;150(5):475-86.
16. Delenclos M, Burgess JD, Lamprokostopoulou A, Outeiro TF, Vekrellis K, McLean PJ. Cellular models of alpha-synuclein toxicity and aggregation. J Neurochem. 2019;150(5):566-76.
17. Puspita L, Chung SY, Shim JW. Oxidative stress and cellular pathologies in Parkinson's disease. Mol Brain. 2017;10(1):1-12.
18. Lazarevic V, Yang Y, Paslawski W, Svenningsson P. α-Synuclein induced cholesterol lowering increases tonic and reduces depolarization-evoked synaptic vesicle recycling and glutamate release. npj Park Dis. 2022;8(1):1-10.
19. Morris HR, Spillantini MG, Sue CM, Williams-Gray CH. The pathogenesis of Parkinson's disease. Lancet. 2024;403(10423):293-304. doi:10.1016/S0140-6736(23)01478-2
20. Tofaris GK. Initiation and progression of α-synuclein pathology in Parkinson's disease. Cell Mol Life Sci. 2022;79(4):210. doi:10.1007/s00018-022-04240-2
21. Chong W, Jiménez J, McIIvin M, Saito MA, Kwakye GF. α-Synuclein enhances cadmium uptake and neurotoxicity via oxidative stress and caspase-activated cell death mechanisms in a dopaminergic cell model of Parkinson's disease. Neurotox Res. 2017;32(2):231-46.

22. Hu Q, Hong M, Huang M, Gong Q, Zhang X, Uversky VN, et al. Age-dependent aggregation of α-synuclein in the nervous system of gut-brain axis is associated with caspase-1 activation. Metab Brain Dis. 2022;37(5):1669-81.
23. Menšíková K, Matěj R, Colosimo C, Rosales R, Tučková L, Ehrmann J, et al. Lewy body disease or diseases with Lewy bodies? npj Park Dis. 2022;8(1):1-12.
24. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. npj Park Dis. 2022;19(2):170-8.
25. Fares MB, Jagannath S, Lashuel HA. Reverse engineering lewy bodies: How far have we come and how far can we go? Nat Rev Neurosci. 2021;22(2):111-31.
26. Giusto E, Yacoubian TA, Greggio E, Civiero L. Pathways to Parkinson's disease: A spotlight on 14-3-3 proteins. npj Park Dis. 2021;7(1):1-10.
27. Werner MH, Olanow CW. Parkinson's disease modification through Abl kinase inhibition: An opportunity. Mov Disord. 2022;37(1):6-15. doi:10.1002/mds.28858
28. Wu R, Chen H, Ma J, He Q, Huang Q, Liu Q, et al. C-Abl-p38α signaling plays an important role in MPTPinduced neuronal death. Cell Death Differ. 2016;23(3):542-52.
29. De Santis S, Monaldi C, Mancini M, Bruno S, Cavo M, Soverini S. Overcoming resistance to kinase inhibitors: The paradigm of chronic myeloid leukemia. Onco Targets Ther. 2022;15:103-16. doi:10.2147/OTT.S289306
30. Pagan FL, Wilmarth B, Torres-Yaghi Y, Hebron ML, Mulki S, Ferrante D, et al. Long-term safety and clinical effects of nilotinib in Parkinson's disease. Mov Disord. 2021;36(3):740-9.
31. Pagan FL, Hebron ML, Wilmarth B, Torres-Yaghi Y, Lawler A, Mundel EE, et al. Nilotinib effects on safety, tolerability, and potential biomarkers in Parkinson disease: A phase 2 randomized clinical trial. JAMA Neurol. 2020;77(3):309-17.
32. Pagan FL, Hebron ML, Wilmarth B, Torres-Yaghi Y, Lawler A, Mundel EE, et al. Pharmacokinetics and pharmacodynamics of a single dose Nilotinib in individuals with Parkinson's disease. Pharmacol Res Perspect. 2019;7(2):1-10.
33. Waller EK. The role of sargramostim (rhGM-CSF) as immunotherapy. Oncologist. 2007;12(S2):22-6. 34. Gendelman HE, Zhang Y, Santamaria P, Olson KE, Schutt CR, Bhatti D, et al. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. npj Parkinsons Dis. 2017;3(1):1-10.
35. Olson KE, Namminga KL, Lu Y, Schwab AD, Thurston MJ, Abdelmoaty MM, et al. Safety, tolerability, and immune-biomarker profiling for year-long sargramostim treatment of Parkinson's disease. EBioMedicine. 2021;67:1-12.
36. Lan AP, Chen J, Zhao Y, Chai Z, Hu Y. mTOR signaling in Parkinson's disease. NeuroMolecular Med. 2017;19(2):1-12.
37. Zhang G, Yin L, Luo Z, Chen X, He Y, Yu X, et al. Effects and potential mechanisms of rapamycin on MPTP-induced acute Parkinson's disease in mice. Ann Palliat Med. 2021;10(3):2889-97.
38. Kalola J, Shah R, Patel A, Lahiri SK, Shah MB. Anti-inflammatory and immunomodulatory activities of Inula cappa roots (Compositae). J Complement Integr Med. 2017;14(3):1-10.
39. Seo JY, Lim SS, Kim J, Lee KW, Kim JS. Alantolactone and isoalantolactone prevent amyloid β25–35 induced toxicity in mouse cortical neurons and scopolamine-induced cognitive impairment in mice. Phytother Res. 2017;31(5):801-11.
40. He D, Liu Y, Li J, Wang H, Ye B, He Y, et al. Isoalantolactone (IAL) regulates neuro-inflammation and neuronal apoptosis to curb pathology of Parkinson's disease. Cells. 2022;11(18):1-15.
41. Ejlerskov P, Hultberg JG, Wang JY, Carlsson R, Ambjørn M, Kuss M, et al. Lack of neuronal IFN-β-IFNAR causes Lewy body- and Parkinson's disease-like dementia. Cell. 2015;163(2):324-39.

42. Tresse E, Riera-Ponsati L, Jaberi E, Sew WQG, Ruscher K, Issazadeh-Navikas S. IFN-β rescues neurodegeneration by regulating mitochondrial fission via STAT5, PGAM5, and Drp1. EMBO J. 2021;40(11):1-12.
43. Hernández-Parra H, Cortés H, Avalos-Fuentes JA, del Prado-Audelo M, Florán B, Leyva-Gómez G, et al. Repositioning of drugs for Parkinson's disease and pharmaceutical nanotechnology tools for their optimization. J Nanobiotechnology. 2022;20(1):1-15.
44. Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Ell P, Soderlund T, et al. Exenatide and the treatment of patients with Parkinson's disease. J Clin Invest. 2013;123(6):2730-40.
45. Kim DS, Choi H, Wang Y, Luo Y, Hoffer BJ, Greig NH. A new treatment strategy for Parkinson's disease through the gut-brain axis: The glucagon-like peptide-1 receptor pathway. Cell Transplant. 2017;26(3):43849.
46. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: A randomized, double-blind, placebo-controlled trial. Lancet. 2017;390(10103):1664-75.
47. Nowell J, Blunt E, Edison P. Incretin and insulin signaling as novel therapeutic targets for Alzheimer's and Parkinson's disease. Mol Psychiatry. 2022;27(3):1472-84.
48. Brundin P, Wyse RK. The linked clinical trials initiative (LCT) for Parkinson's disease. Eur J Neurosci. 2019;49(3):307-15.
49. De Iuliis A, Montinaro E, Fatati G, Plebani M, Colosimo C. Diabetes mellitus and Parkinson's disease: Dangerous liaisons between insulin and dopamine. Neural Regen Res. 2022;17(7):1350-6.
50. Yue X, Li H, Yan H, Zhang P, Chang L, Li T. Risk of Parkinson's disease in diabetes mellitus: An updated meta-analysis of population-based cohort studies. Medicine. 2016;95(18):1-9.
51. Sánchez-Gómez A, Alcarraz-Vizán G, Fernández M, Fernández R, Ezquerra M, Cámara A, et al. Peripheral insulin and amylin levels in Parkinson's disease. Parkinsonism Relat Disord. 2020;72:1-5.
52. Labandeira C, Fraga-Bau A, Arias Ron D, Alvarez-Rodriguez E, Vicente-Alba P, Lago-Garma J, et al. Parkinson's disease and diabetes mellitus: Common mechanisms and treatment repurposing. Neural Regen Res. 2022;17(1):1-10.
53. Cheong JLY, de Pablo-Fernandez E, Foltynie T, Noyce AJ. The association between type 2 diabetes mellitus and Parkinson's disease. J Parkinsons Dis. 2020;10(3):775-89.
54. Craft S, Raman R, Chow TW, Rafii MS, Sun CK, Rissman RA, et al. Safety, efficacy, and feasibility of intranasal insulin for the treatment of mild cognitive impairment and Alzheimer disease dementia: A randomized clinical trial. JAMA Neurol. 2020;77(9):1099-110.
55. Kakkar AK, Singh H, Medhi B. Old wines in new bottles: Repurposing opportunities for Parkinson's disease. Eur J Pharmacol. 2018;837:90-104.
56. Tang Y, Yang K, Zhao J, Liang X, Wang J. Evidence of repurposing drugs and identifying contraindications from real-world study in Parkinson's disease. ACS Chem Neurosci. 2019;10(9):3948-58.
57. Stevens KN, Creanor S, Jeffery A, Whone A, Zajicek J, Foggo A, et al. Evaluation of simvastatin as a disease-modifying treatment for patients with Parkinson's disease. JAMA Neurol. 2022;79(1):71-81.
58. Paudel YN, Angelopoulou E, Piperi C, Shaikh MF, Othman I. Emerging neuroprotective effect of metformin in Parkinson's disease: A molecular crosstalk. Pharmacol Res. 2020;152:104593.
59. Cardoso S, Moreira PI. Antidiabetic drugs for Alzheimer's and Parkinson's diseases: Repurposing insulin, metformin, and thiazolidinediones. Int Rev Neurobiol. 2020;155:37-72.
60. Ping F, Jiang N, Li Y. Association between metformin and neurodegenerative diseases of observational studies: Systematic review and meta-analysis. BMJ Open Diabetes Res Care. 2020;8(1):1-9.

61. Agostini F, Masato A, Bubacco L, Bisaglia M. Metformin repurposing for Parkinson disease therapy: Opportunities and challenges. Int J Mol Sci. 2022;23(2):1-19.
62. Sportelli C, Urso D, Jenner P, Chaudhuri KR. Metformin as a potential neuroprotective agent in prodromal Parkinson's disease-Viewpoint. Front Neurol. 2020;11:1-10.
63. Lin KJ, Wang TJ, Chen SD, Lin KL, Liou CW, Lan MY, et al. Two birds one stone: The neuroprotective effect of antidiabetic agents on Parkinson's disease—Focus on sodium-glucose cotransporter 2 (SGLT2) inhibitors. Antioxidants. 2021;10(4):1-10.
64. Arab HH, Safar MM, Shahin NN. Targeting ROS-dependent AKT/GSK-3β/NF-κB and DJ-1/Nrf2 pathways by dapagliflozin attenuates neuronal injury and motor dysfunction in a rotenone-induced Parkinson's disease rat model. ACS Chem Neurosci. 2021;12(4):674-86.
65. Mui JV, Zhou J, Lee S, Leung KSK, Lee TTL, Chou OHI, et al. Sodium-glucose cotransporter 2 (SGLT2) inhibitors vs. dipeptidyl peptidase-4 (DPP4) inhibitors for new-onset dementia: A propensity score-matched population-based study with competing risk analysis. Front Cardiovasc Med. 2021;8:1-11.
66. Mahoney-Sánchez L, Bouchaoui H, Ayton S, Devos D, Duce JA, Devedjian JC. Ferroptosis and its potential role in the physiopathology of Parkinson's disease. Prog Neurobiol. 2021;196:1-15.
67. Hung AY, Schwarzschild MA. Approaches to disease modification for Parkinson's disease: Clinical trials and lessons learned. Neurotherapeutics. 2020;17(2):436-48.
68. Devos D, Labreuche J, Rascol O, Corvol JC, Duhamel A, Guyon Delannoy P, et al. Trial of deferiprone in Parkinson's disease. N Engl J Med. 2022;386(13):1257-66.
69. Simuni T, Chang Y, Fernandopulle N, Nwabuobi L, Lee H. Isradipine versus placebo in early Parkinson disease: A randomized trial. Ann Intern Med. 2020;172(9):591-8.
70. Venuto CS, Yang L, Javidnia M, Oakes D, Surmeier DJ, Simuni T. Isradipine plasma pharmacokinetics and exposure-response in early Parkinson's disease. Ann Clin Transl Neurol. 2021;8(9):1849-59.
71. Visanji NP, Madan P, Lacoste AMB, Buleje I, Han Y, Spangler S, et al. Using artificial intelligence to identify anti-hypertensives as possible disease-modifying agents in Parkinson's disease. Pharmacoepidemiol Drug Saf. 2021;30(9):1229-36.
72. Tulbă D, Avasilichioaiei M, Dima N, Crăciun L, Bălănescu P, Buzea A, et al. Shared molecular targets in Parkinson's disease and arterial hypertension: A systematic review. Neural Regen Res. 2022;17(1):1-10.
73. Perez-Lloret S, Otero-Losada M, Toblli JE, Capani F. Renin-angiotensin system as a potential target for new herapeutic approaches in Parkinson's disease. Expert Opin Investig Drugs. 2017;26(11):1285-94.
74. Contaldi E, Magistrelli L, Milner A, Cosentino M, Marino F, Comi C. Potential protective role of ACEinhibitors and AT1 receptor blockers against levodopa-induced dyskinesias: A retrospective case-control study. Neural Regen Res. 2021;16(12):2443-51.
75. Udovin L, Otero-Losada M, Bordet S, Chevalier G, Quarracino C, Capani F, et al. Effects of angiotensin type 1 receptor antagonists on Parkinson's disease progression: An exploratory study in the PPMI database. Parkinsonism Relat Disord. 2021;91:1-8.
76. Laudisio A, lo Monaco MR, Silveri MC, Bentivoglio AR, Vetrano DL, Pisciotta MS, et al. Use of ACE inhibitors and falls in patients with Parkinson's disease. Gait Posture. 2017;56:114-9.
77. Jo Y, Kim S, Ye BS, Lee E, Yu YM. Protective effect of renin-angiotensin system inhibitors on Parkinson's disease: A nationwide cohort study. Front Pharmacol. 2022;13:1-12.

78. Bortolanza M, Nascimento GC, Socias SB, Ploper D, Chehín RN, Raisman-Vozari R, et al. Tetracycline repurposing in neurodegeneration: Focus on Parkinson's disease. J Neural Transm. 2018;125(12):1403-15.
79. Sun C, Wang Y, Mo M, Song C, Wang X, Chen S, et al. Minocycline protects against rotenone-induced neurotoxicity correlating with upregulation of Nurr1 in a Parkinson's disease rat model. Biomed Res Int. 2019;2019:1-10.
80. Dominguez-Meijide A, Parrales V, Vasili E, González-Lizárraga F, König A, Lázaro DF, et al. Doxycycline inhibits α-synuclein-associated pathologies in vitro and in vivo. Neurobiol Dis. 2021;151:1-12.
81. Vitola P, Artioli L, Leva S, Balducci C, Forloni G. Rifampicin and its derivative rifampicin quinone reduce microglial inflammatory responses and neurodegeneration induced in vitro by α-synuclein fibrillary aggregates. Cells. 2019;8(8):1-12.
82. Erekat N, Al-Khatib A, Al-Jarrah M. Heat shock protein 90 is a potential therapeutic target for ameliorating skeletal muscle abnormalities in Parkinson's disease. Neural Regen Res. 2014;9(6):616-21.
83. Rane A, Rajagopalan S, Ahuja M, Thomas B, Chinta SJ, Andersen JK. Hsp90 Co-chaperone p23 contributes to dopaminergic mitochondrial stress via stabilization of PHD2: Implications for Parkinson's disease. Neurotoxicology. 2018;65:166-73.
84. Socias SB, González-Lizárraga F, Avila CL, Vera C, Acuña L, Sepulveda-Diaz JE, et al. Exploiting the therapeutic potential of ready-to-use drugs: Repurposing antibiotics against amyloid aggregation in neurodegenerative diseases. Prog Neurobiol. 2018;162:17-36.
85. Acuña L, Hamadat S, Corbalán NS, González-lizárraga F, Dos-santos-pereira M, Rocca J, et al. Rifampicin and its derivative rifampicin quinone reduce microglial inflammatory responses and neurodegeneration induced in vitro by α-synuclein fibrillary aggregates. Cells. 2019;8(8):1-12.
86. Yurtsever İ, Üstündağ ÜV, Ünal İ, Ateş PS, Emekli-Alturfan E. Rifampicin decreases neuroinflammation to maintain mitochondrial function and calcium homeostasis in rotenone-treated zebrafish. Drug Chem Toxicol. 2022;45(4):1544-51.
87. Kaur B, Prakash A. Ceftriaxone attenuates glutamate-mediated neuro-inflammation and restores BDNF in MPTP model of Parkinson's disease in rats. Pathophysiology. 2017;24(2):71-9.
88. Zhou X, Lu J, Wei K, Wei J, Tian P, Yue M, et al. Neuroprotective effect of ceftriaxone on MPTP-induced Parkinson's disease mouse model by regulating inflammation and intestinal microbiota. Oxid Med Cell Longev. 2021;2021:1-12.
89. Kadri H, Lambourne OA, Mehellou Y. Niclosamide, a drug with many (re)purposes. ChemMedChem. 2018;13(11):1088-91.
90. Barini E, Miccoli A, Tinarelli F, Mulholland K, Kadri H, Khanim F, et al. The anthelmintic drug niclosamide and its analogues activate the Parkinson's disease-associated protein kinase PINK1. ChemBioChem. 2018;19(5):425-9.
91. Goulding SR, Lévesque M, Sullivan AM, Collins LM, O'Keeffe GW. Quinacrine and Niclosamide promote neurite growth in midbrain dopaminergic neurons through the canonical BMP-Smad pathway and protect against neurotoxin and α-synuclein-induced neurodegeneration. Mol Neurobiol. 2021;58(7):3405-16.

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