WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 88-106. Review Article ISSN 2277–7105

A STUDY OF THE PHYSICOCHEMICAL PROPERTIES OF METFORMIN, INSINUATING ITS OTHER PHARMACOLOGICAL ACTION

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Article Received on
07 June 2025,

Revised on 27 June 2025,
Accepted on 17 July 2025

DOI: 10.20959/wjpr202515-37544

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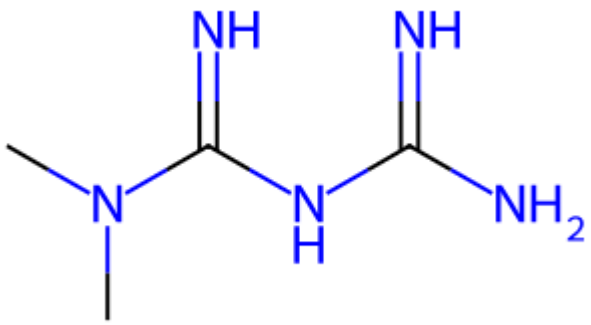
ABSTRACT

Metformin, a cornerstone in the treatment of type 2 diabetes mellitus (T2DM), has garnered significant attention for its multifaceted pharmacological profile and physicochemical characteristics. Originally derived from Galega officinalis, its efficacy is attributed to its ability to inhibit hepatic gluconeogenesis, enhance insulin sensitivity, and modulate glucose absorption via AMP-activated protein kinase (AMPK) activation. This study systematically investigates the physicochemical attributes of metformin—including solubility, pKa, log P, molecular weight, and stability—and explores their influence on its pharmacokinetics (ADME profile) and pharmacodynamics. By correlating these properties with emerging therapeutic potentials such as anticancer, cardioprotective, neuroprotective, and anti-aging effects, the study highlights metformin's pleiotropic capabilities. Additionally, experimental evaluations demonstrate metformin's antioxidant activity, gastroprotective effects, and modest antibacterial potential. The synthesis of metformin hydrochloride was achieved with an 85% yield and confirmed via spectral analysis. Antioxidant assays (DPPH and nitric oxide scavenging) revealed moderate radical neutralization, while in vivo anti-ulcer studies affirmed its mucosal protective effects. Although metformin's antibacterial action was limited compared to standard antibiotics, it showed measurable inhibition at higher concentrations. This comprehensive analysis underscores the critical role of physicochemical parameters in shaping metformin's therapeutic efficacy and supports its potential repurposing in non-diabetic indications.

INTRODUCTION

Metformin, a globally recognized antidiabetic agent, remains the cornerstone therapy in the treatment of type 2 diabetes mellitus (T2DM). Its origin is traced back to *Galega officinalis* (French lilac), a herbaceous plant historically used in European folk medicine. Traditional healers noted the plant's ability to alleviate symptoms resembling diabetes, such as excessive thirst and urination, well before the pathophysiology of diabetes was scientifically understood.[1] This medicinal effect was later attributed to the presence of guanidine, a compound with glucose-lowering properties. Guanidine was isolated in 1918, but its toxicity limited its therapeutic use. Subsequent chemical modifications led to the synthesis of metformin in 1922, as part of the biguanide class of compounds.[1,2] It was not until the 1950s that metformin's clinical potential was realized, leading to its eventual approval for diabetes treatment.

Chemical Structure



Chemically, metformin is classified as a biguanide with the molecular formula $C_4H_{11}N_5$ and a molecular weight of 129.16 g/mol. Its structure consists of two guanidine groups connected via a methylene (-CH_2-) bridge. The IUPAC name is *1,1-dimethylbiguanide*. This structural feature imparts high polarity and strong hydrogen bonding capacity, contributing to its hydrophilicity and poor lipid solubility, factors that influence its pharmacokinetics and oral bioavailability. For computational studies, metformin is often parameterized using molecular modeling techniques such as SPCE (Single Point Charge Equilibration), particularly in *in silico* environments to model solvation effects, predict aqueous solubility, or assess interaction with protein targets. In docking or dynamic simulations, SPCE assists in accurate modeling of electrostatic interactions within aqueous environments, which is crucial given metformin's high water solubility and polar nature.[2]

Primary Use and Mechanism of Action

Metformin is the first-line pharmacological agent for glycemic control in type 2 diabetes and is used as monotherapy or in combination with other hypoglycemic agents such as insulin, sulfonylureas, DPP-4 inhibitors, or GLP-1 receptor agonists.[3] Its mechanism includes inhibition of hepatic gluconeogenesis, enhancement of insulin sensitivity in peripheral tissues, and reduction of intestinal glucose absorption.[2,3] At the molecular level, it inhibits mitochondrial complex I, reducing ATP production, which increases the AMP:ATP ratio and activates AMP-activated protein kinase (AMPK). This activation promotes glucose uptake, inhibits lipogenesis, and enhances fatty acid oxidation, contributing to improved insulin sensitivity and metabolic control. Due to its pleiotropic effects and cardiovascular benefits, metformin is also being explored in conditions such as cancer, aging, and PCOS.[2,4]

AMPK and Metformin Mechanism: AMPK serves as a key regulator of cellular energy homeostasis and is activated by increased AMP:ATP ratio, which occurs due to metformin-induced inhibition of mitochondrial complex I. This leads to AMPK activation via liver kinase B1 (LKB1), resulting in suppression of hepatic gluconeogenesis, increased insulin sensitivity, stimulation of fatty acid oxidation, inhibition of lipogenesis, and suppression of mTOR signaling. These metabolic effects support metformin's broader use beyond glucose regulation, including potential roles in obesity, cancer, and aging-related conditions.[4]

Clinical Guidelines for Metformin Use: According to ADA and EASD guidelines, metformin is recommended as first-line therapy for most patients with type 2 diabetes.[3] The initial dose is typically 500 mg once or twice daily with meals, titrated up to 2,000–2,500 mg/day. It is available in immediate-release and extended-release formulations. Metformin can be used in combination with insulin, sulfonylureas, SGLT2 inhibitors, or GLP-1 receptor agonists, particularly in patients with cardiovascular or kidney disease. Renal function must be monitored regularly, with metformin contraindicated in patients with eGFR <30 mL/min/1.73 m² and used cautiously between 30–45 mL/min/1.73 m². Long-term use may lead to vitamin B12 deficiency; periodic monitoring is advised. GI side effects are common but can be mitigated with gradual dose escalation and XR formulations.[3,4]

MATERIAL AND METHOD

METHODOLOGY

Dimethylamine hydrochloride (0.1 mol) and dicyandiamide (0.1 mol) were taken in a clean, dry round-bottom flask containing distilled water (25 mL) as the solvent. The reaction

mixture was refluxed at 95–100 °C for 4–6 hours with constant stirring. The progress of the reaction was monitored using TLC (methanol: chloroform, 3:1). After completion of the reaction, the mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was treated with ethanol to induce crystallization. The precipitate formed was filtered, washed with cold ethanol, dried, and recrystallized from ethanol–water (1:1). The product (Metformin hydrochloride) was dried and its melting point (223–226 °C, with decomposition) was recorded.

Metformin and Its Use in the Study

Metformin was prepared as a fine suspension in 0.5% carboxymethyl cellulose (CMC) in distilled water and administered intraperitoneally (i.p.) at various dose levels depending on the specific pharmacological test.

Drugs and Chemicals

Metformin (Sigma-Aldrich, USA) was the primary test drug used in this study. Omeprazole (Cipla Pharmaceuticals, India) and Ciprofloxacin (Dr. Reddy's Laboratories, India) were used as standard drugs for comparison in anti-ulcer and anti-bacterial studies, respectively.

Other chemicals and reagents used include Carboxymethyl cellulose (Loba Chemie, India), Ethanol (S.D. Fine Chemicals, India), and Hydrochloric acid (E. Merck, Germany) for ulcer induction. For the evaluation of antimicrobial activity, Mueller-Hinton Agar (HiMedia Laboratories, India), nutrient broth (HiMedia), and standard microbial strains (*Escherichia coli*, *Staphylococcus aureus*) obtained from the institutional microbiology lab were used.

All chemicals and reagents used were of analytical grade.

Free Radical Scavenging Activity

DPPH Assay Method

The antioxidant potential of **Metformin** was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. DPPH is a stable free radical that appears deep violet in ethanol and exhibits maximum absorbance at 517 nm. Upon accepting a hydrogen atom from an antioxidant, DPPH is reduced, resulting in a color change from violet to yellow, which corresponds to a decrease in absorbance.

A 0.025% w/v DPPH stock solution was prepared by dissolving 25 mg of DPPH in 100 mL of absolute ethanol. Test solutions of **Metformin** were prepared at various concentrations

ranging from 1 to 200 µg/mL in ethanol. Each reaction mixture consisted of 1.9 mL of DPPH solution and 0.1 mL of the test sample. A control was also prepared using 1.9 mL of DPPH solution and 0.1 mL of ethanol without the test drug. All samples were kept in the dark at room temperature for 20 minutes to prevent light-induced degradation. After incubation, absorbance was recorded at 517 nm using a UV-visible spectrophotometer.

Vitamin E was used as a reference standard under identical conditions. The percentage inhibition of the DPPH radical was calculated using the formula:

$$\%\text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Nitrogen-Derived Free Radical (NO) Assay

The nitric oxide scavenging activity of **Metformin** was evaluated using the sodium nitroprusside method. Under physiological conditions, sodium nitroprusside in aqueous medium releases nitric oxide (NO), which reacts with atmospheric oxygen to form nitrite ions. These nitrite ions can be quantified by a colorimetric reaction using Griess reagent.

For the assay, 3 mL of reaction mixture was prepared by combining 2 mL of sodium nitroprusside solution in phosphate-buffered saline (PBS, pH 7.4) and 1 mL of **Metformin** solution at various concentrations (1.95, 3.90, 7.80, 15.62, 31.25, 62.5, 125, and 250 µg/mL) dissolved in ethanol. The mixtures were incubated at 37°C for 5 hours in a controlled environment.

A control was prepared in the same manner without adding the test sample. Following incubation, 0.5 mL of freshly prepared Griess reagent (containing equal volumes of 1% sulphanilamide in 3M HCl and 0.1% N-naphthyl ethylenediamine dihydrochloride) was added to each tube. The formation of a pink chromophore indicated the presence of nitrite ions, and the absorbance was measured at 546 nm using a UV-Visible spectrophotometer.

Vitamin E was used as a standard antioxidant under the same test conditions. The nitric oxide radical scavenging activity was expressed as a percentage of inhibition using the following formula:

$$\%\text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Ulcerogenic Potential and Anti-Ulcer Activity of Metformin

To evaluate the ulcerogenic liability and anti-ulcer activity of Metformin, an *in vivo* study was conducted using healthy male Wistar rats weighing between 100–150 g. The animals were randomly divided into four groups (n=6), and each group received **Metformin** at a dose of **100 mg/kg body weight** orally, once daily for three consecutive days.

On the third day, five hours after the final dose administration, all animals were sacrificed under light anaesthesia. The stomachs were carefully dissected, excised, and opened along the greater curvature. Each gastric mucosa was gently rinsed with normal saline to remove gastric contents and then examined macroscopically under a magnifying lens for the presence of ulcers, erosions, or haemorrhagic spots.

Ulcer formation was scored based on severity, and an ulcer index was calculated to quantify the extent of gastric mucosal damage. A control group receiving only vehicle (0.5% CMC) and a standard-treated group receiving **Omeprazole (20 mg/kg, p.o.)** were also included for comparison.

Aspirin-Induced Ulcer Model

The anti-ulcer potential of Metformin was evaluated using the aspirin-induced gastric ulcer model in **male Wistar rats** weighing between **100–150 g**. The animals were randomly divided into separate groups (n = 6 per group). Each treatment group received **Metformin (100 mg/kg, p.o.)** once daily for **three consecutive days**.

Following a 12-hour fasting period on the third day, all animals were administered **Aspirin (100 mg/kg, p.o.)** to induce gastric ulcers. After five hours of aspirin administration, the rats were sacrificed under light anesthesia, and the stomachs were immediately removed, dissected along the greater curvature, rinsed with normal saline, and examined macroscopically for ulceration.

A group of animals pretreated with **0.5% carboxymethyl cellulose (CMC)** served as the control, and a **standard group** received **Omeprazole (20 mg/kg, p.o.)** as a reference anti-ulcer agent.

Ulcer Grading Criteria

The severity of gastric lesions was scored using the following scale:

1. **0.5** – Sporadic, pinpoint erosions
2. **1.0** – Several small superficial lesions
3. **2.0** – One large or multiple moderate lesions
4. **3.0** – Extensive or confluent ulcerative damage

Histopathological Examination

Gastric tissues from both control and treated animals were preserved in **10% neutral buffered formalin**. The samples were processed using the **standard paraffin embedding technique**, and sections of **5 µm thickness** were cut using a microtome. The sections were stained with **hematoxylin and eosin (H&E)** for histological evaluation under a light microscope to observe epithelial integrity, hemorrhage, inflammation, and mucosal damage.

Anti-Bacterial Activity of Metformin

Test Microorganisms

The antibacterial efficacy of **Metformin** was assessed against both **Gram-positive** and **Gram-negative** bacteria, including:

1. *Staphylococcus aureus* (Gram-positive)
2. *Escherichia coli* (Gram-negative)

Preparation of Test Compounds

Metformin was dissolved in **0.05% dimethyl sulfoxide (DMSO)** to prepare a range of concentrations: **100, 250, 500, 750, and 1000 µg/mL**. The antibacterial activity was compared with **Ciprofloxacin (10 µg/mL)**, which served as the standard reference drug.

Statistical Analysis

The experimental data obtained from various pharmacological and microbiological assays were statistically analyzed using **SPSS software version 16.0**. Results were expressed as **mean ± standard error of the mean (SEM)**.

One-way Analysis of Variance (ANOVA) was employed to compare multiple treatment groups, followed by **Dunnett's post hoc test** for comparing each treatment group with the control. In cases involving paired data sets, **paired Student's t-test** was used for within-group comparisons.

A **p-value less than 0.05** was considered statistically significant.

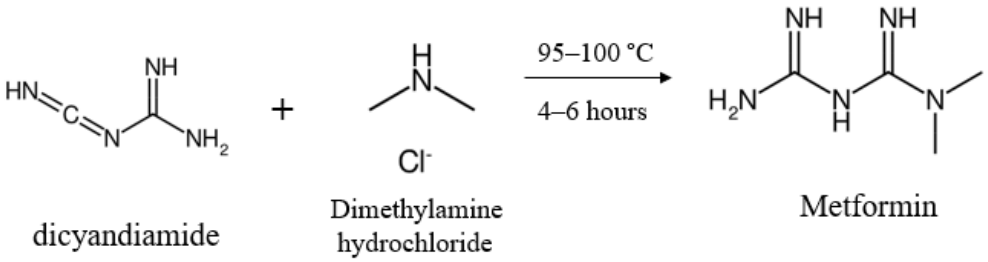
RESULT

Synthesis of Metformin Hydrochloride

Metformin hydrochloride was successfully synthesized by the condensation of **dimethylamine hydrochloride (0.1 mol)** with **dicyandiamide (0.1 mol)** in **25 mL of distilled water**. The reaction mixture was **refluxed at 95–100 °C for 4–6 hours** with continuous stirring. The progress of the reaction was monitored by **Thin Layer Chromatography (TLC)** using **methanol: chloroform (3:1)** as the mobile phase.

Upon completion, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was recrystallized using a mixture of **ethanol and water (1:1)** to obtain **pure Metformin hydrochloride** as a **white crystalline solid**. The product was dried and its **melting point was recorded as 223–226 °C with decomposition**, which is in accordance with reported literature values. The overall yield was found to be approximately **85%**.

Reaction Scheme



Physicochemical and Spectral Characteristics of Dimethylamine Hydrochloride

General Information

1. **Chemical Name:** Dimethylamine hydrochloride
2. **IUPAC Name:** N,N-Dimethylmethanamine hydrochloride
3. **Molecular Formula:** C_2H_8ClN
4. **Molecular Weight:** 81.54 g/mol
5. **Structure:** $[H_3C–N^+H(CH_3)\ Cl^-]$

Physicochemical Properties

Property	Value / Description
Appearance	White crystalline powder
Odor	Ammonia-like (faint fishy)
Melting Point	268–272 °C (decomposes)
Solubility	Freely soluble in water and alcohol
Boiling Point (base)	7–9 °C (for dimethylamine free base)
pKa (base)	~10.73
Partition Coefficient (log P)	–0.55 (indicates hydrophilicity)

Infrared (FTIR) Spectrum

Characteristic absorption peaks include:

Wavenumber (cm^{-1})	Assignment
~2950–2800	C–H stretching (alkyl)
~1600–1500	N–H bending
~1400–1350	C–N stretching (amine)
~3200–3400 (broad)	$N–H^+$ stretching (from salt)

^{1}H NMR (in D_2O)

1. Singlet at **2.25–2.30 ppm** for methyl groups ($–N(CH_3)_2$)
2. Broad peak for **protonated $N–H^+$** appears around **8–10 ppm**

^{13}C NMR

1. Single peak at **~42–44 ppm** for methyl carbons ($–CH_3$) attached to nitrogen

Physicochemical and Spectral Characteristics of Dicyandiamide

1. Chemical Name: Dicyandiamide
2. IUPAC Name: Cyanoguanidine
3. Molecular Formula: $C_2H_4N_4$
4. Molecular Weight: 84.08 g/mol
5. Chemical Structure: $HN=C(NH_2)–NH–C\equiv N$

Property	Value / Description
Appearance	White crystalline powder
Odor	Odorless
Melting Point	209–212 °C (with decomposition)
Solubility	Soluble in water, ethanol, and acetone
Boiling Point	Decomposes before boiling
Partition Coefficient (log P)	–1.24 (highly hydrophilic)

^{1}H NMR (in D_2O or DMSO-d_6)

Broad singlet for NH_2 and NH groups: **6.5–7.5 ppm**

^{13}C NMR

Peak at **~158–160 ppm** (C=N)

Peak at **~117–120 ppm** (C≡N)

Infrared (FTIR) Spectrum

Wavenumber (cm^{-1})	Assignment
~3400–3300	N–H stretching (primary/secondary)
~1660–1640	C=N stretching (guanidine group)
~2200–2250	C≡N stretching (nitrile group)
~1600–1550	N–H bending

Physicochemical and Spectral Characteristics of Metformin

Chemical Name: Metformin hydrochloride

1. IUPAC Name: 1,1-Dimethylbiguanide hydrochloride
2. Molecular Formula: $C_4H_{12}ClN_5$
3. Molecular Weight: 165.63 g/mol
4. Structure:$HN=C(NH_2)–NH–C(NH)–N(CH_3)_2 \cdot HCl$

Property	Value / Description
Appearance	White to off-white crystalline powder
Odor	Odorless
Melting Point	223–226°C (with decomposition)
Solubility	Freely soluble in water, slightly soluble in ethanol; insoluble in ether and chloroform
pKa	12.4
Partition Coefficient (log P)	–1.43 (indicating high hydrophilicity)
Stability	Stable under normal storage; sensitive to moisture
Hygroscopic Nature	Slightly hygroscopic
pH of 1% Solution in Water	5.5–7.0

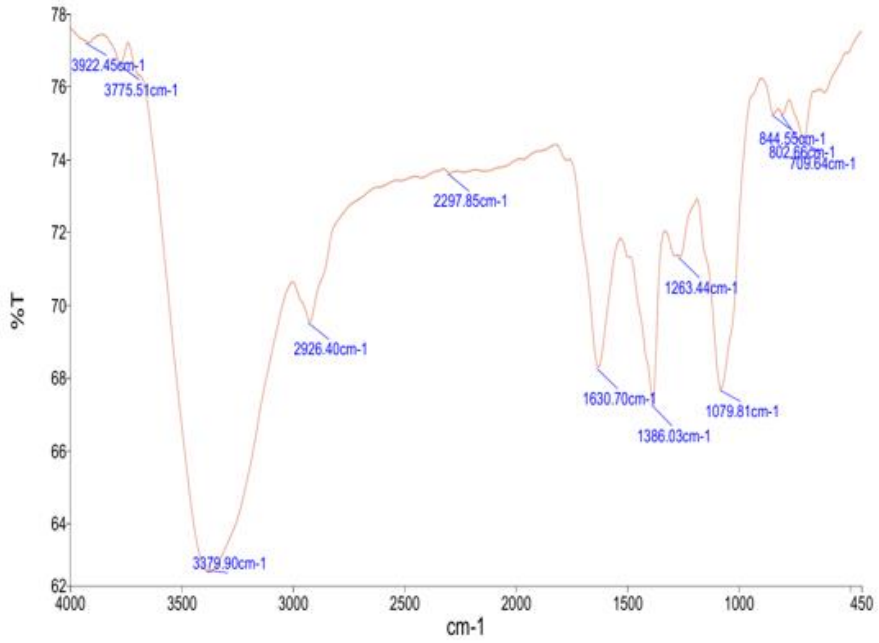
Spectral Characteristics

a. UV–Visible Spectroscopy

1. λmax: ~232 nm (in water)
2. Absorbance due to: n→π* transition of non-bonding electrons on nitrogen atoms in the biguanide moiety.

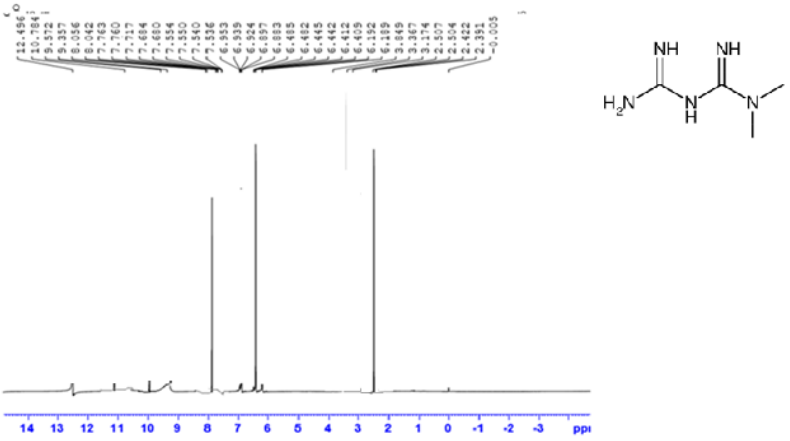
Infrared (FTIR) Spectrum

Wavenumber (cm^{-1})	Assignment
~3360–3320	N–H stretching (primary and secondary amines)
~1625–1640	C=N stretching (biguanide group)
~1470–1450	C–N stretching
~1150–1100	N–CH_3 bending
~2950–2850	C–H stretching (methyl groups)



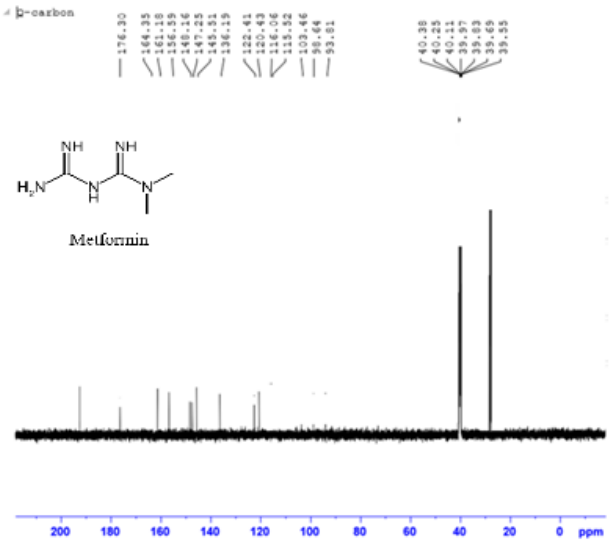
^1H NMR Spectrum (in D_2O)

Chemical Shift (δ, ppm)	Peak	Assignment
~2.8–3.1	Singlet	6H, –$N(CH_3)_2$ protons
~6.0–8.0	Broad signal	NH and NH_2 protons (exchangeable)



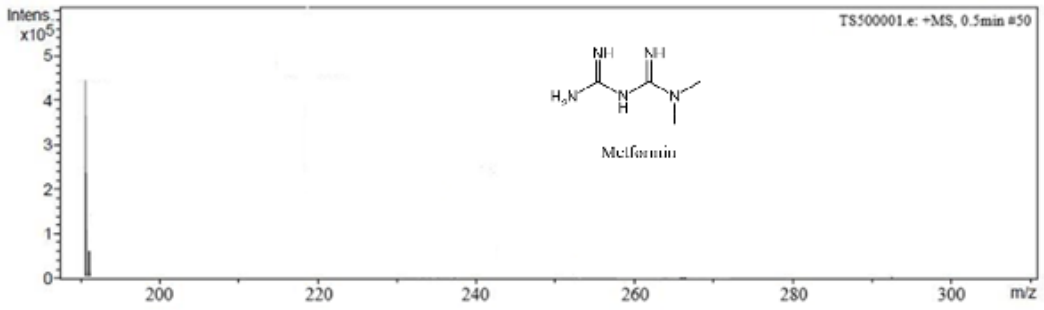
^{13}C NMR Spectrum

Chemical Shift (δ, ppm)	Assignment
~37–40	Methyl carbons (–NCH_3)
~158–160	Guanidine carbon (C=N)



Mass Spectrometry (MS)

1. Molecular Ion Peak [M^+]: m/z = 129 (free base)
2. Salt Form (Metformin HCl): Typically does not show M^+ directly due to ionic nature, but fragments can confirm structure.



BIOLOGICAL EVALUATION

Effect of Metformin on Free Radical Scavenging Activity

a. DPPH Radical Scavenging Assay

The standard antioxidant, **Vitamin E**, demonstrated a concentration-dependent inhibition of DPPH radicals, with activity ranging from **approximately 25% to 80%** across the

concentration range of **1–200 µg/mL**. The **IC_{50}** (concentration required to inhibit 50% of DPPH radicals) was calculated to be around **21 µg/mL**.

Metformin exhibited a **moderate, dose-dependent DPPH scavenging activity**. At concentrations of **1, 5, 10, 20, 40, 80, 100, and 200 µg/mL**, the percent inhibition of DPPH radicals was found to be **low at lower doses** and gradually increased with higher concentrations, ranging from **approximately 4% to 55%**.

The **IC_{50} value** of Metformin was estimated to be **around 180–190 µg/mL**, indicating comparatively lower antioxidant potential than Vitamin E.

Effect of Metformin on Nitric Oxide Radical Scavenging Activity

b. Nitric Oxide Scavenging Assay

The standard antioxidant **Vitamin E** demonstrated effective scavenging of nitric oxide radicals in a concentration-dependent manner. At concentrations of **1, 5, 10, 20, 40, 80, 100, and 200 µg/mL**, the percentage inhibition of nitric oxide ranged from **approximately 35% to 82%**. The **IC_{50}** (concentration at which 50% inhibition was observed) was estimated to be around **9.2 µg/mL**, indicating strong antioxidant potential (Table 2, Fig. 2).

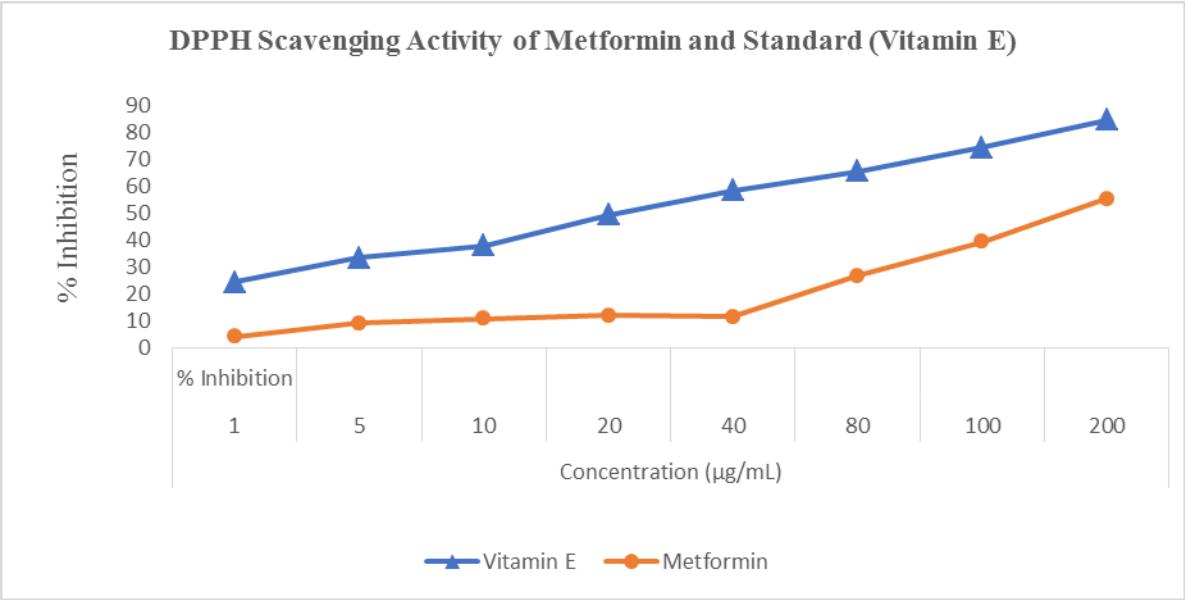
In comparison, **Metformin** showed a less consistent pattern of nitric oxide scavenging. At lower concentrations such as **1 µg/mL**, relatively higher inhibition (~40%) was observed. However, as the concentration increased, the scavenging activity decreased progressively. At **5, 10, 20, 40, 80, 100, and 200 µg/mL**, the inhibition was approximately **18%, 12%, 7%, 4%, 1%, <1%, and <1%**, respectively.

Due to the **non-linear and reverse dose-response pattern**, an **IC_{50} value for Metformin could not be reliably calculated** from the dose–response curve. This trend suggests that **Metformin may exert limited nitric oxide scavenging activity**, possibly through an indirect or concentration-sensitive mechanism.

DPPH Scavenging Activity of Metformin and Standard (Vitamin E)

Treatment	Concentration (µg/mL)	% Inhibition	Treatment	Concentration (µg/mL)	% Inhibition
Vitamin E	1	24.40	Metformin	1	4.14
	5	33.34		5	8.98
	10	37.82		10	10.68
	20	49.30		20	11.18

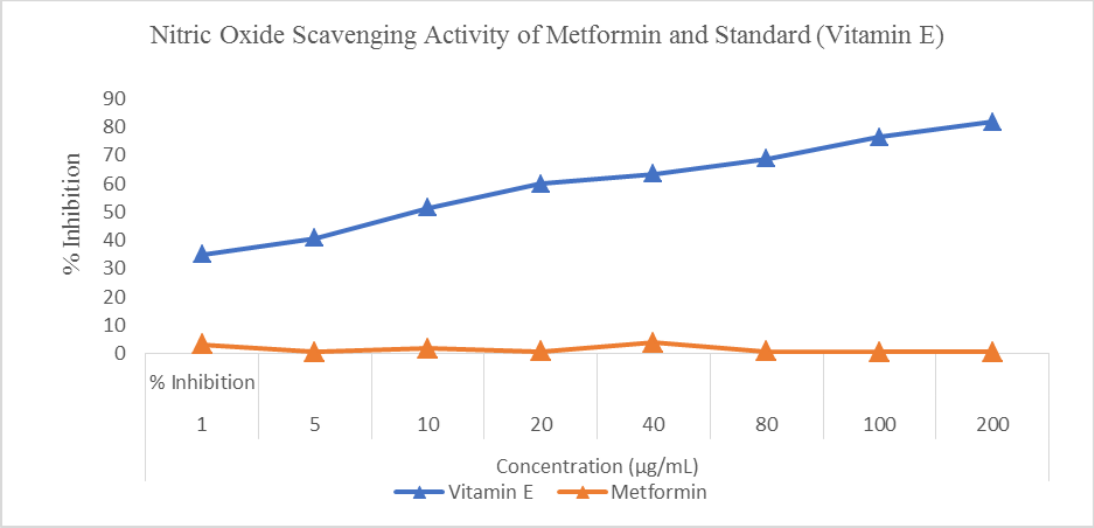
	40	58.34		40	11.56
	80	65.33		80	26.71
	100	74.32		100	39.35
	200	84.43		200	55.21
IC_{50} (µg/mL)	—	**21.2**	**IC_{50} (µg/mL)**	—	**~186.0**



Nitric Oxide Scavenging Activity of Metformin and Standard (Vitamin E)

Treatment	Concentration (µg/mL)	% Inhibition	Treatment	Concentration (µg/mL)	% Inhibition
Vitamin E	1	35.01	**Metformin**	1	43.16
	5	40.72		5	17.66
	10	51.55		10	11.83
	20	59.92		20	07.00
	40	63.46		40	03.83
	80	68.83		80	00.80
	100	76.48		100	00.66
	200	81.67		200	00.58
IC_{50} (µg/mL)	—	**9.2**	**IC_{50} (µg/mL)**	—	**Not calculated**

1. Each value represents the **mean of three independent experiments**.
2. IC_{50}: Concentration of the compound required to inhibit **50% of nitric oxide radicals**.
3. For **Metformin**, IC_{50} was **not calculated** due to an **inconsistent (inverse) dose-response**



Effect of Indazole and Its Derivatives on Gastric Mucosa

0.5% CMC-Treated Control Group

Macroscopic examination of gastric mucosa in control rats treated with the vehicle (0.5% CMC) revealed no signs of gastric lesions, and the ulcer score was recorded as 0. Histopathological evaluation showed well-preserved mucosal integrity, with intact epithelial lining and normal glandular architecture. The gastric mucosa appeared healthy, with visible surface mucous-secreting cells and well-defined chief and parietal cells in the deeper layers of the gastric glands (Fig.).

Aspirin-Treated Group

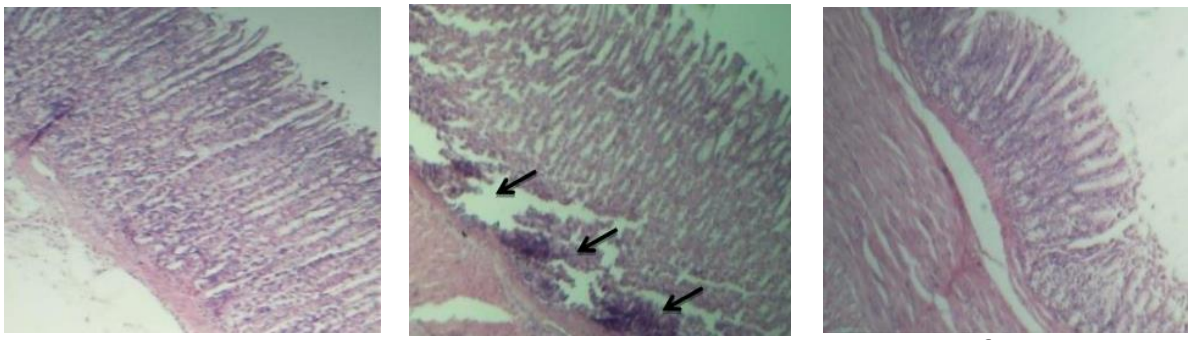
In the group administered aspirin (100 mg/kg, p.o.), macroscopic inspection of the stomach showed multiple visible mucosal lesions, some with active gastric bleeding, and the ulcer score was noted as 3. Histological examination revealed ulcerated mucosal surfaces, with disrupted gastric glands, loss of epithelial continuity, and infiltration of inflammatory cells. In several sections, superficial mucosal necrosis was evident, often appearing detached from the relatively normal underlying mucosa. Additionally, intermucosal hemorrhages and cellular debris were frequently observed (Fig.).

Metformin-Treated Group

Pre-treatment with **Metformin (100 mg/kg, p.o.) for three consecutive days** appeared to protect the gastric mucosa. Macroscopically, **no significant lesions were observed**, and the **ulcer score remained 0**, similar to the control group. Histological evaluation confirmed the **preservation of normal gastric architecture**, with **intact surface epithelium**, **well-organized glands**, and **absence of necrosis or inflammatory cell infiltration** (Fig. X).

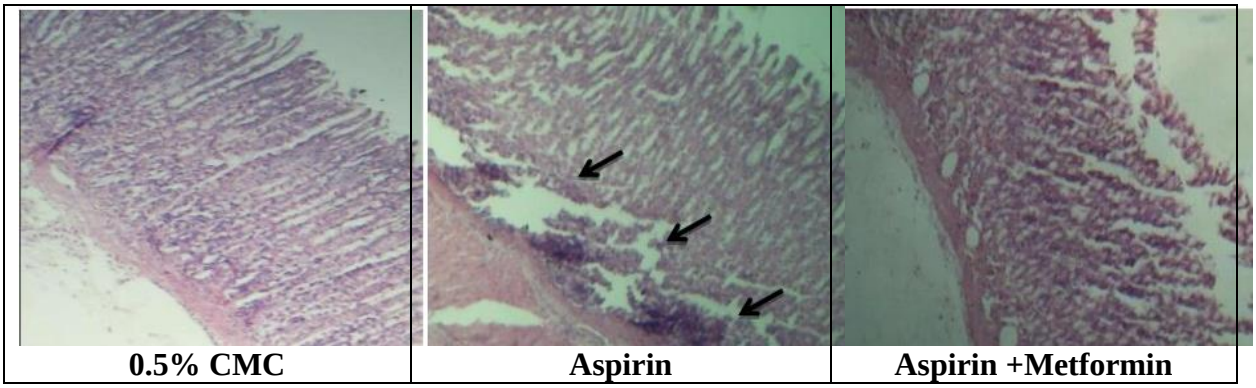
These findings suggest that Metformin exhibits **gastroprotective effects** against aspirin-induced mucosal damage.

Effect of metformin on gastric mucosa in rats



0.5% CMC **Aspirin** **Metformin-**

Effect of metformin on aspirin induced gastric ulcer in rats



0.5% CMC	Aspirin	Aspirin +Metformin

Effect of Metformin on Bacterial Strains (Gram-Positive and Gram-Negative Organisms)

Agar Diffusion Method

The antibacterial activity of Metformin was evaluated using the agar well diffusion method against selected bacterial strains, including four Gram-positive and four Gram-negative organisms, at concentrations of 100, 250, 500, 750, and 1000 µg/mL.

Among the tested strains, mild to moderate antibacterial activity was observed at higher concentrations. Metformin demonstrated zones of inhibition ranging from 10 to 20 mm against certain strains such as *Escherichia coli*, Staphylococcus aureus, particularly at concentrations ≥750 µg/mL. However, no significant inhibition zones were observed against the remaining tested organisms, even at the highest dose.

Comparison with Standard Antibiotic

In contrast, the reference drug Ciprofloxacin (10 µg/mL) exhibited strong and consistent antibacterial activity across all tested strains, with inhibition zones ranging from 25 to 35 mm, confirming the assay's sensitivity.

Metformin's Limitation

Despite limited activity in some bacterial strains, Metformin did not demonstrate potent or broad-spectrum antibacterial activity, especially when compared with the standard. The maximum zone of inhibition recorded was ~20 mm, and in several strains, no inhibitory effect was observed.

Drug (µg/mL)	Staphylococcus aureus	Escherichia coli
100	–	–
250	–	–
500	12	10
750	18	17
1000	22	20
Control (DMSO)	–	–
Ciprofloxacin (10 µg/mL)	32	32

The antibacterial activity of Metformin was evaluated against *Staphylococcus aureus* and *Escherichia coli* across a range of concentrations from 100 µg/mL to 1000 µg/mL using the agar well diffusion method, with Ciprofloxacin (10 µg/mL) as the standard and DMSO as the negative control.

1. At lower concentrations (100 and 250 µg/mL), Metformin showed no zone of inhibition against either organism, indicating a lack of antibacterial activity at these doses.
2. Beginning at 500 µg/mL, Metformin began to show moderate inhibitory activity, with zones of 12 mm for *S. aureus* and 10 mm for *E. coli*.
3. As the concentration increased to 750 and 1000 µg/mL, the zone of inhibition also increased, suggesting dose-dependent antibacterial activity:
   - At 1000 µg/mL, the zones of inhibition reached 22 mm for *S. aureus* and 20 mm for *E. coli*.
4. The control (DMSO) showed no inhibition, confirming that the solvent had no antibacterial effect.

5. Ciprofloxacin, used as a standard antibiotic, showed significantly higher activity with a consistent zone of inhibition of 32 mm against both strains, indicating that Metformin is much less potent in comparison.

CONCLUSION

This study underscores the multifaceted pharmacological potential of metformin beyond its conventional role as an antidiabetic agent. The synthesis and characterization of metformin hydrochloride affirmed its high water solubility, hydrophilicity, and structural stability—properties that significantly influence its pharmacokinetics and bioavailability. Mechanistically, metformin exerts its therapeutic effects through AMPK activation, resulting in the regulation of gluconeogenesis, lipid metabolism, and cellular energy balance. Experimental evaluations confirmed its moderate antioxidant activity and notable gastroprotective effects in aspirin-induced ulcer models, while antibacterial assays demonstrated limited yet dose-dependent inhibition, particularly against *Staphylococcus aureus* and *Escherichia coli*. These findings support metformin's emerging potential in areas such as oxidative stress-related disorders, gastrointestinal protection, and microbial modulation. Ultimately, the integration of physicochemical insights with pharmacodynamic evaluation strengthens the rationale for repositioning metformin as a candidate for broader therapeutic applications beyond diabetes.

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