Synthesis and evaluation of novel Schiff's base Indole derivatives as Anti-inflammatory agents

Divya Singh[1, 2*], Rajeev Kharb[1], Satish Kumar Sharma[3], Vidushi Sharma[4]

[1] Amity Institute of Pharmacy, Amity University, Noida-201313, Uttar Pradesh, India

[2] Sunder Deep Pharmacy College, Dasna, Ghaziabad, Uttar Pradesh, 201009

[3] Glocal School of Pharmacy; Glocal University, Mirza Pur Pole, Saharanpur, Uttar Pradesh-247121

[4] Delhi Pharmaceutical Sciences and Research University (DPSRU), Sec 3, Pushp Vihar, New Delhi 110017

Emails ID:

Divya Singh: tsdivass7@gmail.com
Rajeev Kharb: rajeevkharb_2007@rediffmail.com
Satish Kumar Sharma: satishdipsar55@gmail.com
Vidushi Sharma: vidushimaitry@gmail.com

Address for correspondence
Divya Singh
Associate Professor
Sunder Deep Pharmacy College,
Dasna, Ghaziabad, Uttar Pradesh, 201009
tsdivass7@gmail.com
8506020323

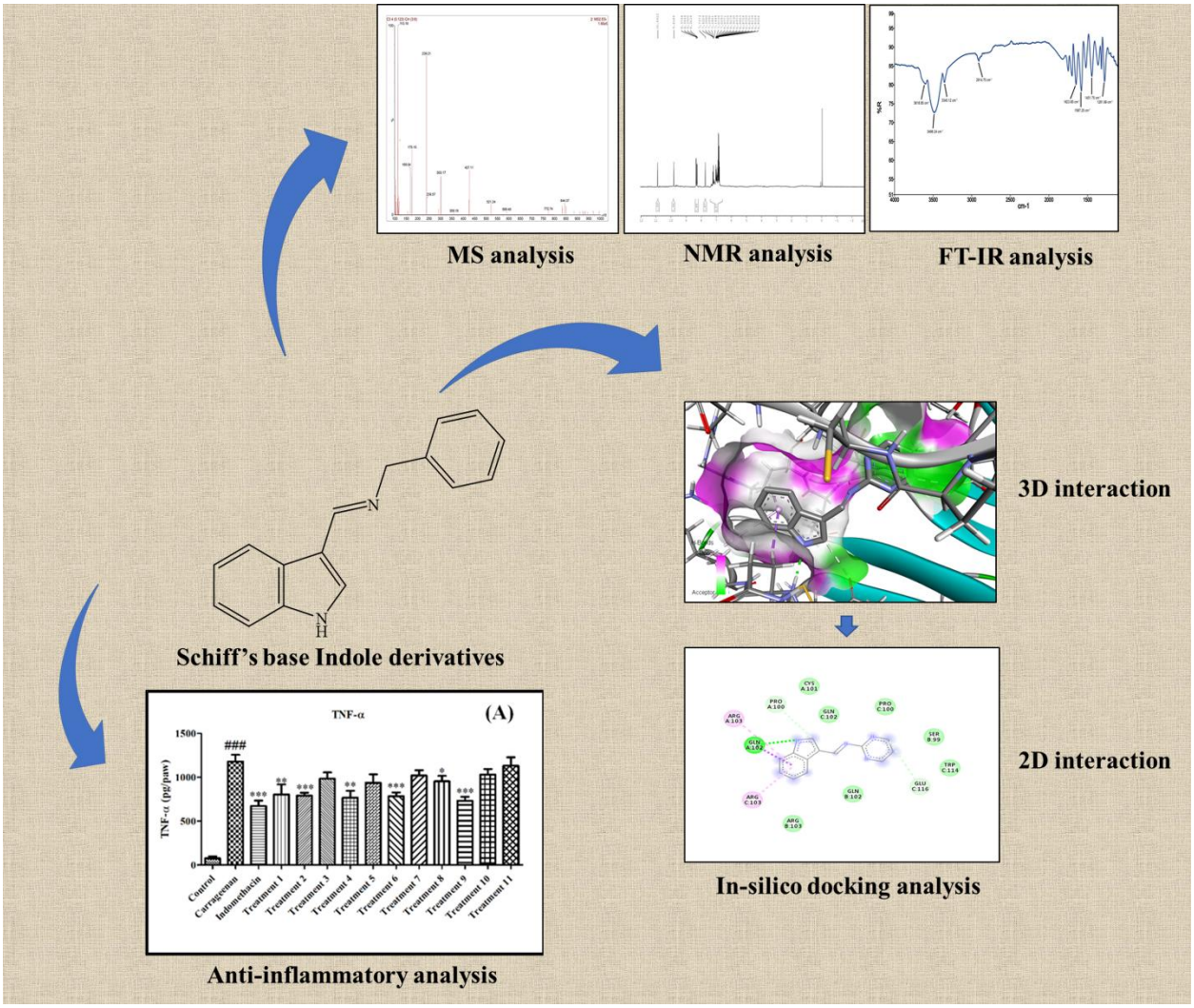
Abstract

Development of novel Schiff's base Indole derivatives as Anti-inflammatory agents is critically needed to obviate the necessity of the potent anti-inflammatory agents. The aim of the study is associated to develop and evaluate Schiff's base Indole derivatives as Anti-inflammatory agents using Schiff's base synthesis approaches. each synthesized compound was characterized spectroscopically. In-silico docking analysis was performed to evaluate the potent anti-inflammatory agents via exploring interaction profile of each compounds with TNF-α protein followed by in-vivo estimation of active components against carrageenan induced paw edema. The outcome of the study showed that among different compounds, the compound such as C1IN, C2IN, C3IN, C7IN, C8IN and C11IN were found most active against COX-2 as well as paw edema induced by carrageenan. Furthermore, it has been showed that C1IN, C2IN, C6IN, C8IN and C9IN were found with significant reduction of inflammatory cytokines such as TNF-α and

IL-1β. Hence, it can be concluded that the developed Schiff's base Indole derivatives can be alternative components as anti-inflammatory agents.

Keywords: Schiff's Base Indole Derivatives, Spectroscopy Analysis, Anti-Inflammatory Activity, In-Silico Docking Study.

Graphical Abstract



1. Introduction

Consistence in anti-microbial resistance is one of the most concerning issue to the healthcare system and researchers to obviate microorganism induced inflammation or associated pathogenesis. Microorganisms has been acknowledged as the tiniest organism that cannot seen with the naked eyes. Microorganisms often exist in three environments: water, soil with the highest density, and the air. There are many different types of microbes, some of which are helpful to people and some of which have a significant negative impact on their health.[1] Also, it is known that the human body is the home to millions of various kinds of bacteria. Most of the pathogenic organism are termed to caused severe onsets in the body even leads to death. Oxidative stress and inflammation are one of the serious morbidities that is caused by microorganism via production of several species of free radical as well as cytokines, respectively.[2]

Inflammation has been generally characterized as the protective action of body against invading of chemicals or metabolic adducts, toxins or noxious stimuli that cause pain, heat inside the body, swelling of affected site, etc. furthermore, it has been demonstrated that inflammation cause numerous morbidities such as cancer, cardiovascular disorders, aging, as well as others life-threatening diseases. It is classified in two forms that is acute inflammation and chronic inflammation. In acute inflammation, overproduction of reactive oxygen as well as nitrogen species, inflammatory mediators, production of pro-inflammatory and activation of enzymes complexes.[3,4] Many more implication of inflammation has been defined as that progressive generation of cytokines represents the chronic inflammation or pathogenesis in the body system. Many sittings have been implicated in response to mimic microbial induced inflammation or management of microorganism induced disease.[5] Development of novel and potent anti-microbial or anti-inflammatory agents is critically needed to obviate the pathogenesis induced by onsets of microorganism.

In modern system of medicines, there are various pharmaceuticals drugs such as 1,3,4-thiadiazole, coumarin, indole, chitosan. thioether-modified 1,3,4-thiadiazole, and imine that have been implicated for treating microbial infection as well as resist their growth. Due to exponential growth in anti-bacterial resistance, development of potential anti-microbial and anti-inflammatory agents is critically needed to treating bacterial onsets and inflammation.

Schiff bases (SBs) are widely used in many areas of chemistry, including analytical, inorganic, and organic chemistry. They are also recommended for use in solar energy applications. They are utilized as dyes, catalysts, polymer stabilizers, luminescence chemosensors, catalyzers in the fixation of CO_2 biolubricant additives, and luminescence chemosensors. The requirement for SB synthesis is further emphasized by a wide range of pharmacological and biological functions, including antimalarial, antiproliferative, analgesic, anti-inflammatory, antiviral, antipyretic, antibacterial, and antifungal activities. It has been used in synthesis of many novel compounds such as indole, imidazole, benzimidazole, etc, and exponentially used in development of novel compounds for targeting the treatment of several acute and chronic ailments.[6]

Considering the factors, the present study is associated to explore the anti-inflammatory effect of synthesized novel Schiff's base Indole derivatives using several in-silico and in-vivo approaches and thus developing effective anti-inflammatory regimen for treatment of inflammation or associated complications.

Material and methods

2.1. Chemicals and reagents

In this study several chemicals such as Chloroaniline, Fluoro aniline, 2,5-Dichloro aniline, Difluoro aniline, 2,5-Dibromo aniline, 3,4,5-trimethoxy aniline, 3,5-Dimethoxyaniline, Methoxy aniline (Anisidine), Tert-butylamine, Ethylene Diamine, 3,5-Bis(Trifluoromethyl)benzyl amine, Benzyl amine, 4-amino-2-chloro benzonitrile, Thiophene-2-ethylamine, 3-Amino Pyrazole, 1-Methyl-3-Amino Pyrazole, 2-Amino Pyrimidine, 2-aminophenol and 2,4-dimethylaniline were used and purchased from Sisco Research Laboratories Pvt. Ltd. India as well as Sigma-Aldrich Chemicals Pvt. Ltd.

2.2. Chemistry for the synthesis of Schiff's base Indole derivatives

In the synthesis of indole Synthesis of Schiff's base Indole derivatives, initially indole-3-carboxaldehyde was synthesized. In brief, gramine methiodide[1-(1H-indol-3-yl)-N,N,N-trimethylmethaminium iodide] (348 mg) and DMF (4ml), NaNO2 (239mg) was added and the obtained solution was stirred at room temperature for 6 hours using magnetic stirrer. Thereafter, 15 ml of distilled water was added and the solution was extracted with 20 ml of water and dried Na_2SO_4, the solution was evaporated to get Indole-3-carboxaldehyde. The reaction was monitored by TLC for completion of reaction and characterized by IR, NMR and LCMS

interpretation. Thereafter, in another step, a equimolar quantities (0.6mmol) of indole-3-carboxaldehyde and primary amine with one amino group was dissolved in methanol (10ml). The reaction mixture was heated at 70^0 C in reflux condenser with stirring for 1 - 2 hours. The reaction mixture was monitored by using the technique of Thin Layer Chromatography on silica gel G plates by using toluene: ethyl acetate: Formic acid (6:4:1, v/v/v) as solvent system. The solid obtained was cooled, filtered and purified by re-crystallization from methanol (22). The schematic representation for synthesis of Schiff's base Indole derivatives has been represented in Figure 1.

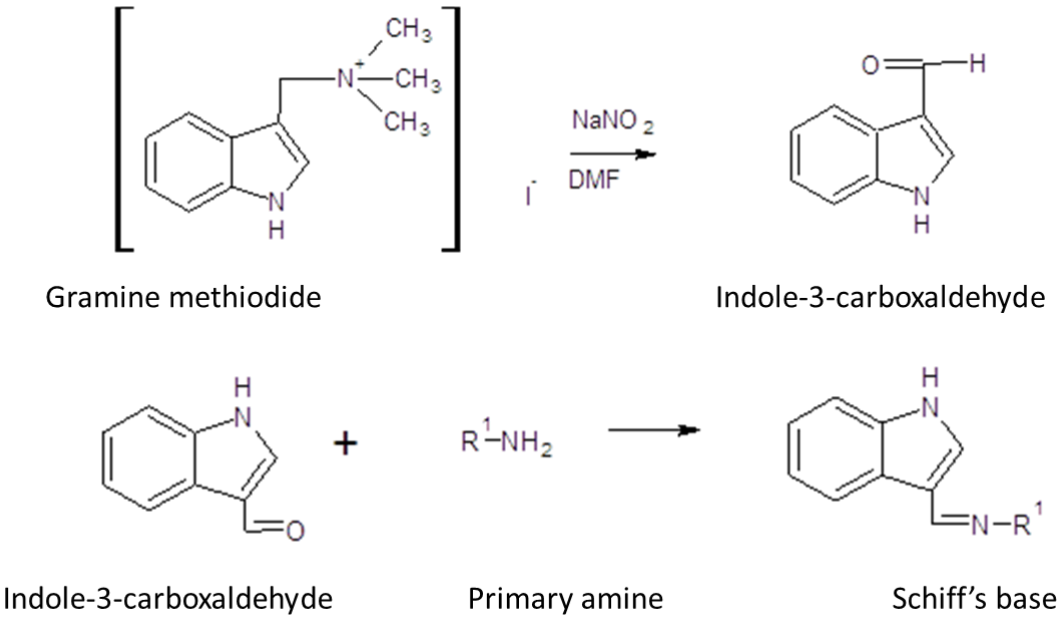
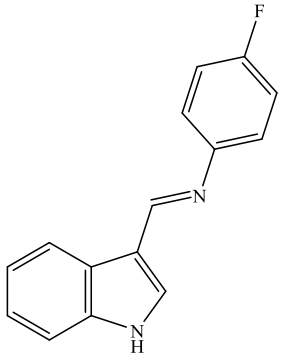
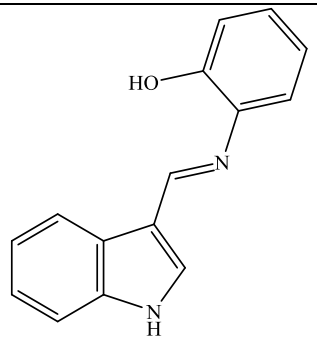


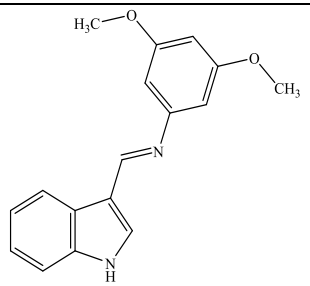
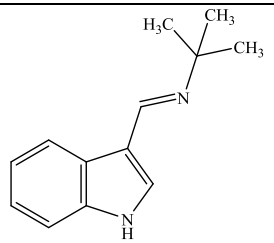
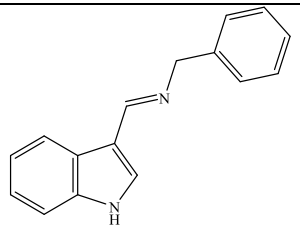
Figure 1: Schematic representation for synthesis of Schiff's base Indole derivatives.

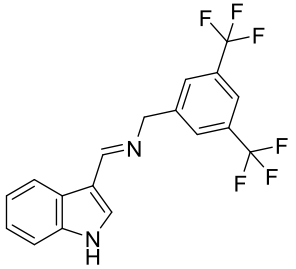
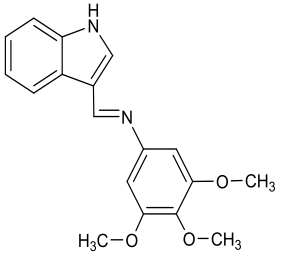
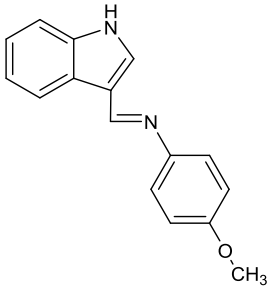
2.3. Characterization and screening of therapeutically active compounds

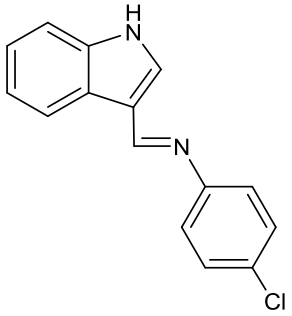
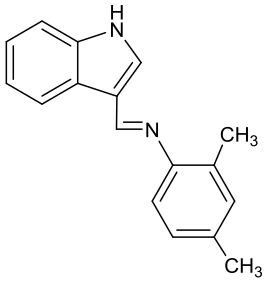
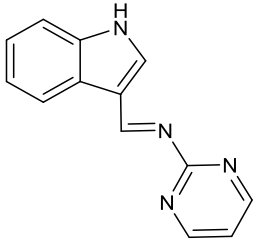
In this study, different spectroscopical approaches such as mass spectroscopy (MS), Schiff's base Indole derivatives (FT-IR), Nuclear Magnetic Resonance (NMR) have been used for characterization and identification of synthesized compounds as per the referenced protocols with some modifications.[7] Furthermore, in-silico analysis was performed to determine the therapeutically active components based on their interaction with Tumor necrosis factor alpha (TNF-α; grid dimension of the protein center_x = -3.641, center_y = 68.631, center_z = 131.508, size_x = 53, size_y = 53, size_z = 53) and the data has been represented in Figure 2. The characterized components have been represented in Table 1 and spectroscopical outcomes of therapeutically active compounds have been represented as follows.

Table 1: Synthesized indole derivatives, their structure and molecular docking interaction profile with TNF- α

S. No.	**Compound code**	**Indole derivatives (Compound name)**	**Chemical structure**	**Molecular weight**	**Interaction profile with TNF-α**		
					Affinity (kcal/mol)	**RMSD l.b**	**RMSD u.b.**
1.	C1IN	(*E*)-*N*-(4-fluorophenyl)-1-(1*H*-indol-3-yl)methanimine		238.27	-7.6	3.426	7.085
2.	C2IN	2-[(*E*)-(1*H*-indol-3-ylmethylidene)amino]phenol		236.27	-7.6	2.097	6.628

3.	C3IN	(*E*)-*N*-(3,5-dimethoxyphenyl)-1-(1*H*-indol-3-yl)methanimine		280.33	-6.5	31.083	32.738
4.	C4IN	*N*-*tert*-butyl-1-(1*H*-indol-3-yl)methanimine		200.29	-6.5	1.857	2.809
5.	C5IN	*N*-benzyl-1-(1*H*-indol-3-yl)methanimine		234.30	-7.1	2.667	5.981

6.	C6IN	*N*-[3,5-bis(trifluoromethyl)benzyl]-1-(1*H*-indol-3-yl)methanimine		370.30	-7.6	2.240	2.910
7.	C7IN	(*E*)-1-(1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)methanimine		310.35	-5.7	0.674	2.672
8.	C8IN	(*E*)-1-(1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)methanimine		250.30	-5.9	25.430	26.910

9.	C9IN	(*E*)-*N*-(4-chlorophenyl)-1-(1*H*-indol-3-yl)methanimine		254.06	-5.9	24.689	26.233
10.	C10IN	(*E*)-*N*-(2,4-dimethylphenyl)-1-(1*H*-indol-3-yl)methanimine		248.33	-6.4	8.132	8.925
11.	C11IN	(*E*)-1-(1*H*-indol-3-yl)-*N*-(1-methyl-1*H*-pyrazol-3-yl)methanimine		222.25	-6.6	2.792	6.122

Spectroscopical characterization of compounds

(E)-N-(4-fluorophenyl)-1-(1H-indol-3-yl)methanimine (C1IN)

MS (ESI) m/z: 239.21 (M+1H); (FTIR, υmax, cm−1); 3612.99, 3425.14, 3321.22 (=N, -NH and fluorine stretching), 2934.95 (CH) 1624.77 (C=C/-C=N), 1458.42 (stretching of C-H), 1005.47 (aliphatic amines, aromatic C-H vibrations). ^{1}H-NMR (CD3OD, 500MHz): δ 7.086 (4H, m, fluorophenyl ring), 7.224, 7.723, 8.304 and 10.902 (3H, 2d,s, Ar-CH-7, 2, 4 and NH of indole ring), 9.801, (1H, s, C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 115.323, 116.634 (2C, indole ring at 3rd and 7th position), 119.34, 126.738 (4C of fluorophenyl ring), 122.36, 124.784 (3C at indole ring at 4th, 5th and 6th position), 163.764 (1C of C=N bonding).

2-[(E)-(1H-indol-3-ylmethylidene)amino]phenol (C2IN)

MS (ESI) m/z: 237.16 (M+1H); (FTIR, υmax, cm−1); 3618.86, 3498.24, 3345.12 (=N, -NH and OH), 2914.75 (CH) 1623.85, 1587.25, 1451.75 (C-N, C=C/C-H bonding), 1261.99 (aliphatic amines or aromatic C-H vibrations). ^{1}H-NMR (CD3OD, 500MHz): δ 6.972 (6H, m, 6CH of benzene rings of phenol and indole), 7.4988, (1H, d, Ar-CH of indole ring at 7th position), 7.232 and 8.401(2H, s,m, 2CH at 2nd and 4th position of indole ring), 10.902 and 9.801, (2H, 2s, NH and C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 115.323, 116.634 (2C, indole ring at 3nd and 7th position), 154.763 (C-OH), 124.376 (2H, of indole ring at 4th and 6th position), 163.37 (C=N).

(*E*)-*N*-(3,5-dimethoxyphenyl)-1-(1*H*-indol-3-yl)methanimine (C3IN)

MS (ESI) m/z: 281.24 (M+1H); (FTIR, υmax, cm−1); 3518.42, 3387.55 (=N, -NH), 2989.44 (CH_3), 1623.73 (C=C), 1698.03 (C=O), 1517.43, 1408.42 (C-O-C and C-H), 1179.32 (aliphatic amines aromatic C-H vibrations). ^{1}H-NMR (CD3OD, 500MHz): δ 3.682 (6H, s, 2CH_3), 6.3001, (3H, s, Ar-CH of dimethoxyphenyl), 6.9422 (3H, m, Ar-3CH of indole ring at 5th, 6th, and 7th position), 7.701, and 8.4001 (2H, s,d, CH of indole ring at 3rd and 4th position), 10.902 and 9.801, (2H, 2s, NH and C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 59. 352 (2C of dimethoxyphenyl group), 101.347, 104.932 (3C, of dimethoxyphenyl ring at 2nd, 4th and 6th position), δ 115.376, 116.688 (2C, indole ring at 3nd and 7th position), 121.376, 124.463 (2C of indole ring at 4th, 5th and 6th position).

(*E*)-1-(1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)methanimine (C7IN)

MS (ESI) m/z: 333.31 ($M+Na^+$); (FTIR, υmax, cm−1); 3651.42, 3381.37 (=N, -NH), 2982.46 (CH_3) 1832.47, 1749.52, 1609.48 (C=C/-C=N, C-O), 1247.52 (C-O-C and C-H). ^{1}H-NMR (CD3OD, 500MHz): δ 3.542 and 3.804 (9H, s, $3CH_3$), 6.211, (2H, s, Ar-2CH of trimethoxyphenyl), 6.983 (3H, m, Ar-3CH of indole ring at 5th, 6th, and 7th position), 7.731, and 8.403 (2H, s,d, CH of indole ring at 2nd and 4th position), 10.903 and 9.802, (2H, 2s, NH and C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 59.63, 63.252 (2C, $3OCH_3$ of trimethoxyphenyl group), 104.63 (2C, of trimethoxyphenyl ring at 2nd and 6th position), 114.78, 115.84 (2C at 3rd and 7th position of indole ring) 163.36 (1C of C=N)

(*E*)-1-(1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)methanimine (C8IN)

MS (ESI) m/z: 251.24 (M+1H); (FTIR, υmax, cm−1); 3415.43, 3287.96 (=N, -NH), 2917.53, 2834.82 (CH_3/CH_2) 1633.23 (C=C/-C=N), 1253.73 (C-O-C and C-H). ^{1}H-NMR (CD3OD, 500MHz): δ 3.543 (3H, s, CH_3), 6.481, (2H, s, Ar-2CH of methoxyphenyl at 3rd and 4th position), 6.985 (3H, m, Ar-3CH of indole ring at 5th, 6th, and 7th position), 7.611, 8.009, 8.429 (4H, 2d,s, 2CH of methoxyphenyl at 2nd and 6th position at 2nd and 4th position of indole ring), 11.003 and 9.802, (2H, 2s, NH and C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 59.345 (1C of methoxy group), 114.634, 115.437 (2C, at 3rd and 7th position of indole ring), 118.376, 125.892 (4C at 3rd, 5th and 2nd and 6th position of methoxyphenyl ring), 163.276 (1C for C=N group).

(*E*)-1-(1*H*-indol-3-yl)-*N*-(1-methyl-1*H*-pyrazol-3-yl)methanimine (C11IN)

MS (ESI) m/z: 223.18 (M+1H); (FTIR, υmax, cm−1); 3441.42, 3357.98 (=N, -NH), 3085.47, 2967.48 (C-H-C) 1653.49, 1609.73 (C=C/-C=N). ^{1}H-NMR (CD3OD, 500MHz): δ 6.985 (3H, m, Ar-3CH of indole ring at 5th, 6th, and 7th position), 6.274, 8.426 and 8.872 (5H, 2m,d, 2CH of methoxyphenyl at 2nd and 4th position of indole ring and 3CH of pyrazol ring), 11.023 and 9.322, (2H, 2s, NH and C=N). ^{13}C-NMR (CD3OD, 500MHz): δ 114.327, 115.234 (2C, at 3rd and 7th position of indole ring), 122.76, 124.478 (3C at 4th, 5th and 6th of indole ring). 161.783, 163.89, 165.302 (3C at 1st, 3rd, 5th pyrazol ring and C=N).

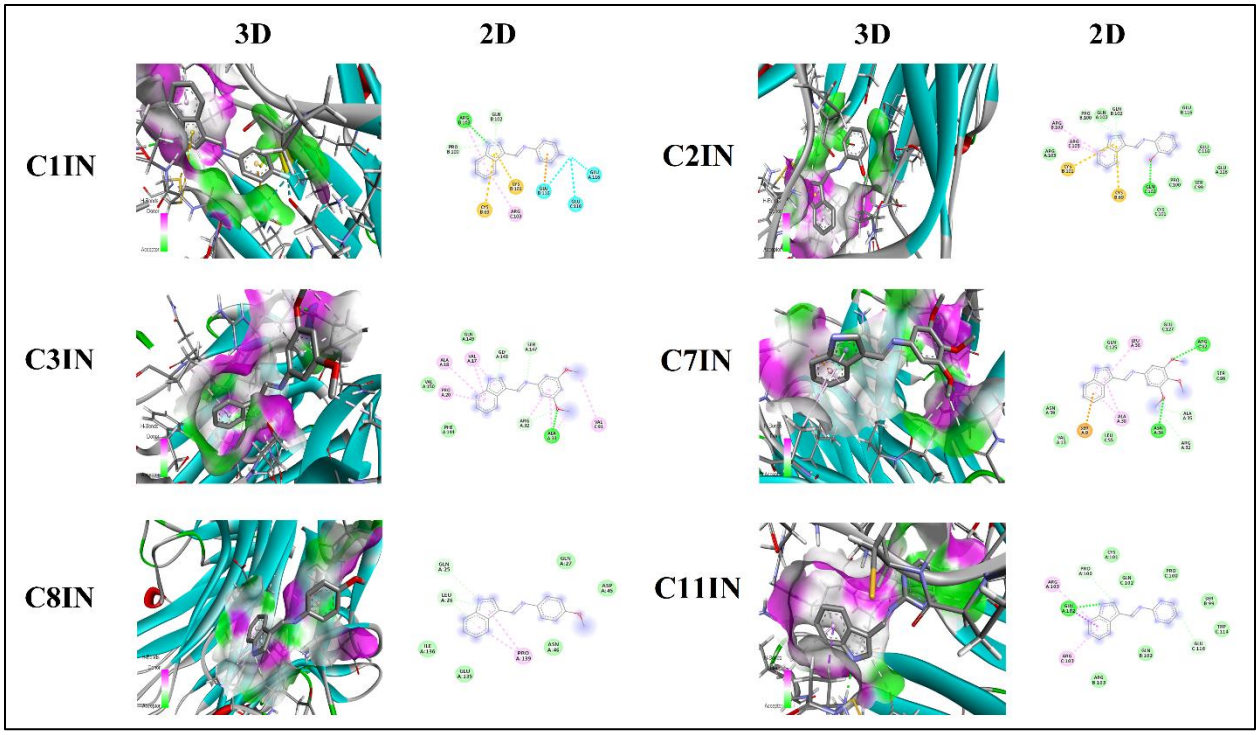


Figure 2: In-silico docking interaction profile of selective components with TNF-α protein.

2.4. In-vivo anti-inflammatory activity

Anti-inflammatory activity of Schiff's base derivatives was performed using carrageenan induced paw edema method. The selection or screening of compounds for in-vivo anti-inflammatory activity has been made based on the in-silico study outcomes. The derivatives which exhibited significant interaction with the targeted protein were selected for the in-vivo study. However, some of the compounds which exhibited least interaction, were also included for in-vivo screening to demonstrate that whether the activity of the compounds in directly proportional to the biological interaction or binding energy of the compounds. Based on this consideration, a total of eleven indole Schiff's base derivatives were selected for in-vivo study.

2.4.1. Experimental animals

The *in-vivo* experimental studies were carried out on *Wistar albino* rats obtained from Amity Institute of Pharmacy, approved by the Institutional Animal Ethics Committee with approval number (163/PO/RE/S/12/CPCSEA/2021-6). The animals used for the experimental study were weighed successively with an average weight of 190 ± 20 g. All the animals were housed in polypropylene cages and habituated to regular laboratory conditions with light and dark cycles of

12:12 h, temperature: 23 ± 2°C; and relative humidity 55 ± 5%. The animals were fed a standard pellets diet and delivered unrestricted access to normal saline (ad libitum) throughout experimentation. The studies were performed under the stringent guiding principle of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC) of Amity Institute of Pharmacy, Gurugram.

2.4.2. Anti-inflammatory activity

2.4.2.1. Carrageenan-induced rat paw edema model

The rats were divided into nine groups containing six rats **(Tabe 2)** and inflammation was induced according to edema assay. Paw edema acute inflammation was induced in a right hind paw of each rat by intraplantar injection of 100 µl of 1%, (w/v) (suspension in saline) lambda carrageenan. 300 mg/kg of each drug was administered to each rate for evaluation of the protectove effect against infammation induced by carrageenan. Paw edema volume was measured before and after carrageenan injection at the 30, 60, 120, 180, 240 and 300 minutes, using a plethysmometer. Indomethacin was used as a standard drug. After 300 min, the animals were sacrificed and the carrageenan-induced edema feet were dissected and stored at − 80°C. [8 9].

Table 2: Treatment schedule of the animals.

S. No.	**Groups**	**Treatment group for Indole derivatives**	**Treatment shedule**	**Animals required**
1.	Group 1	Control group	Animals received normal saline and chew diet	6
2.	Group 2	Toxic group	Animals received intraplantar injection of 100 µl of 1%, (w/v)	6
3.	Group 3	Standard group (Indomethacin: 5 mg/kg)	Animals received Indomethacin: 5 mg/kg, intraperitoneally	6
4.	Group 4	Treatment 1 (C1IN)	Drug (300 mg/kg) + Carrageenan	6
5.	Group 5	Treatment 2	Drug (300 mg/kg) + Carrageenan	6

		(C2IN)		
6.	Group 6	Treatment 3 (C3IN)	Drug (300 mg/kg) + Carrageenan	6
7.	Group 7	Treatment 4 (C4IN)	Drug (300 mg/kg) + Carrageenan	6
8.	Group 8	Treatment 5 (C5IN)	Drug (300 mg/kg) + Carrageenan	6
9.	Group 9	Treatment 6 (C6IN)	Drug (300 mg/kg) + Carrageenan	6
10.	Group 10	Treatment 7 (C7IN)	Drug (300 mg/kg) + Carrageenan	6
11.	Group 11	Treatment 8 (C8IN)	Drug (300 mg/kg) + Carrageenan	6
12.	Group 12	Treatment 9 (C9IN)	Drug (300 mg/kg) + Carrageenan	6
13.	Group 13	Treatment 10 (C10IN)	Drug (300 mg/kg) + Carrageenan	6
14.	Group 14	Treatment 11 (C11IN)	Drug (300 mg/kg) + Carrageenan	6

2.4.2.2. Measurements of pro-inflammatory markers

Pro-inflammatory markers in blood were determined using the standard protocol with some modifications as followed. After the last paw measurement, all the animals were anesthetized with an injection of ketamine hydrochloride 80-90 mg/ kg and sacrificed for collection of blood samples for the analysis of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF-α), and interleukin- 1ß (IL-1ß) levels by ELISA kit purchased from Abbkine (China) [10].

In brief, blood obtained samples were kept undisturbed to coagulate at room temperature for 60 minutes and then centrifuged at 13000 rpm for 15 minutes. The obtained crude serum samples were collected and placed into the new tubes and stored at −20°C for further analysis [9].

Statistical analysis

Data are represented statistically as Mean ± SD (n=6) for evaluation of significant difference and the variability among the data. In statistical analysis, the data was represented using One-way and Two way ANOVA followed by tukey test. the statistical difference was made based on the p-value summary and p-value. The stistically significant values were considred as *p-value < 0.05.

Results

Schiff's base Indole derivatives were synthesized, characterized successfully. The potential active components were determined by in-silico docking analysis and further evaluated for their anti-inflammatory activity against carrageenan induced paw edema in wistar rats. The outcome of the study has been follows as.

2.5. Anti-inflammatory activity of the synthesized compounds

Anti-inflammatory activity of synthesized compounds was performed successfully against carrageenan induced paw edema. The findings showed that the effect of each drug was found significant (*$p<0.05$) as compared to the group treated with carrageenan. However, it can be suggested that out of six examined compounds of indole derivative, the compounds C1IN, C2IN and C8IN were found to be exhibited significant protective effect against inflammation induced by carrageenan in paw hind. The effect of these two compounds were better comparable to the effect of standard drug which is used as indomethacin. However, the effect of each treated compounds was found significant as compared to the toxic group. Moreover, the study suggests that compound C1IN, C2IN and C8IN can be the promising agent against varieties of inflammatory cytokine which are responsible for inflammation. The outcome of the study has been represented in figure 2.

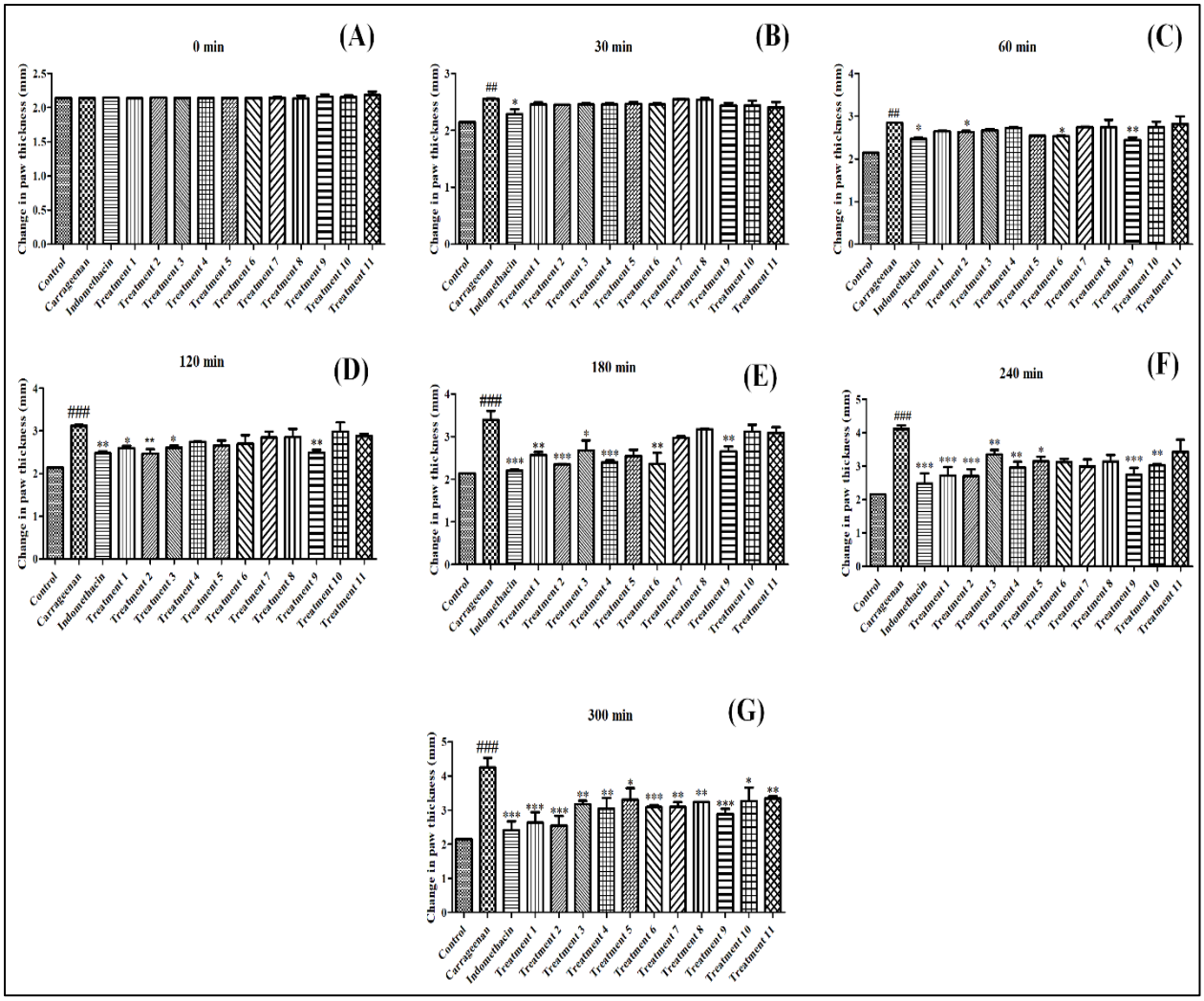


Figure 3: Anti-inflammatory activity of indole derivatives against carrageenan induced paw edema. Data are expressed as mean ± SD (n = 6). Values with superscripts (#) is significantly different between normal control vs toxic control and superscripts (*) is significantly different between control vs treatment groups. The value $p < 0.05$ considered significant. The symbol represents the significance level such as #/* ($p < 0.05$); ##/** ($p < 0.01$) and ###/*** ($p < 0.001$).

2.6. Assessment of inflammatory cytokines in blood serum

Inflammation has been generally acknowledged as the immune response against varieties of the endogenous and exogenous stimulus that generally cause several pathophysiological changes in the body via production of different varieties of the cytokines. The drugs that are active against the cytokines or reduces the production of cytokines, has been generally characterized as the Anti-inflammatory drugs. Hence, the study aimed to evaluate the anti-inflammatory effect

through assessment of the inflammatory cytokines such as TNF-α and IL-1β. The assessment of the inflammatory cytokine was performed in the plasma separated from the blood of the treated rats. The findings of the study showed that among the different compounds of indole Schiff base derivatives, compounds C1IN, C2IN, C6IN, C8IN and C9IN are the most prominent compounds which are active against the inflammatory cytokines and significantly (<0.05) reduces the cytokines level and act against toxicity induced by carrageenan. The effect of test drug was found comparable then the effect of the standard drug. The outcome of the study has been summarized in the figure 3.

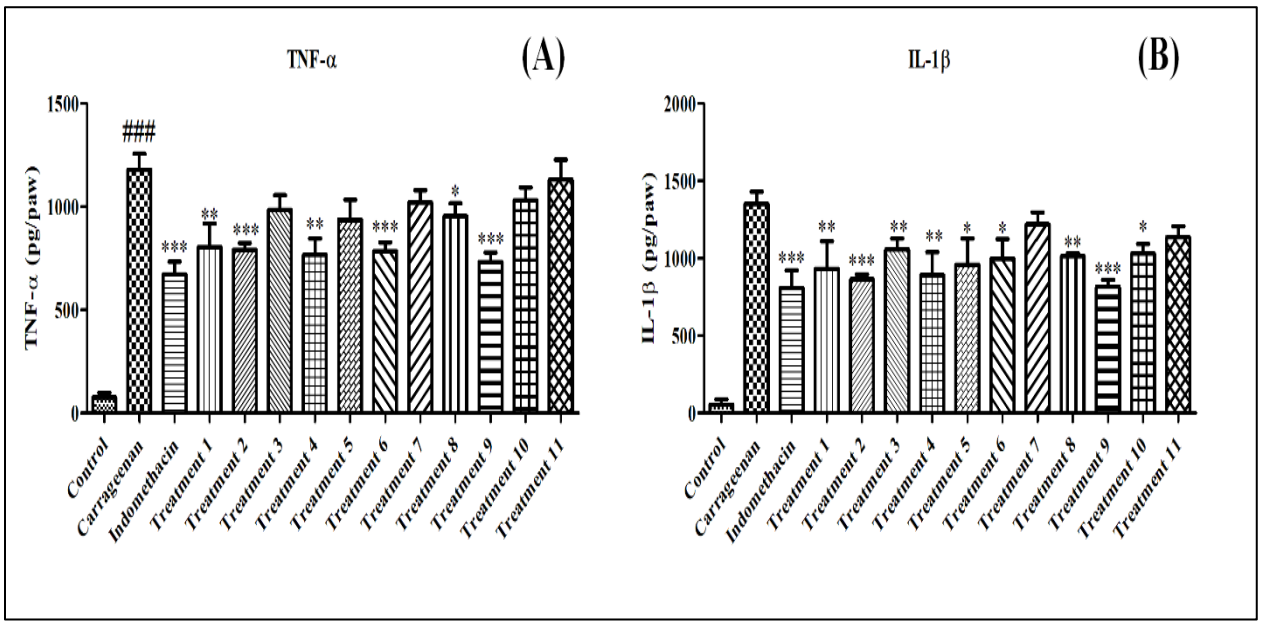


Figure 4: *Anti-inflammatory effect of indole derivatives against carrageenan induced inflammation. Figure (A) represents protective effect of indole derivatives against TNF-α while figure (B) represent the effect against IL-1β. The data are expressed as mean ± SD (n = 6). Values with superscripts (#) is significantly different between normal control vs toxic control and superscripts (*) is significantly different between control vs treatment groups. The value p < 0.05 considered significant. The symbol represents the significance level such as #/* (p < 0.05); ##/** (p < 0.01) and ###/*** (p < 0.001).*

3. Discussion

Carrageenan induced paw edema is one of the most conventional method for assessing the targeted compounds exhibits anti-inflammatory activity. The model is used to consider the most

precise and liable method or model for drug induced inflammation. Several studies has been conducted to determine the effect of natural or even synthesized components against inflammation induced by carrageenan.[11–13]

Bhatt *et al*., synthesized a new series of indole derivatives and evaluated for their ulcerogenic activity, anti-inflammatory activity, lipid peroxidation, analgesic activity, ulcer index and cyclooxygenase expression activities. The compounds were characterized spectroscopically. According to the study's findings, three synthetic substances (S3, S7, and S14) significantly reduced inflammation when compared to the reference medicine indomethacin. Cyclooxygenase (COX) expression activity and an ulcerogenic index were present in compound S3. It was supplying the stomach sparing function while specifically suppressing the production of COX-2. These substances may be able to bind to the COX-2 enzyme, according to docking studies. Between the OH of Tyr 355 and the NH2 of Arg 120 with the carbonyl group, compound S3 produced a hydrogen bond that resembled the one indomethacin forms. This work sheds light on substance S3, a new lead molecule that functions as a selective COX-2 inhibitor and anti-inflammatory.[14]

Ibrahim *et al*. also designed, synthesized, and produced new non-steroidal anti-inflammatory drugs (NSAIDs) that were evaluated using spectroscopic techniques such 1H NMR, 13C NMR, IR, and MS. Using the Carrageenan-induced paw edema model and Diclofenac as a control substance, the compounds were created by the Schiff's condensation reaction. Their anti-inflammatory activity was then examined. The percentage of edema inhibition showed that all substances had similar anti-inflammatory effect as diclofenac. Additionally, molecular docking research was used in virtual screening to verify the anti-inflammatory efficacy. Intriguingly, compound M2 had the strongest binding energy score (-10.765) and the lowest virtual screening docking score (-12.142), as well as the highest in vivo activity (61.32% inhibition) when compared to normal Diclofenac (51.36% inhibition).[15]

Inflammation is triggering response to the immune response against the onsets of various stimulus weather that may be a chemical induced or drug induce immune response or pathogenic induced response. Furthermore, it helps to identify the pathophysiological condition via assessing the different varieties of cytokines such as TNFs, ILs, NF-kB etc.[16] Furthermore, anti-inflammatory effect of synthesized compounds was determined against carrageenan induced paw

edema. Cytokine level in the blood serum was determined and the outcome of the study showed that compounds C1IN, C2IN, C6IN, C8IN and C9IN were found most active and potentiate against induced cytokine level. It significantly reduced the level of cytokines and reduces the possibility of inflammations.

According to Takada *et al*., ndole-3-carbinol impeded NF-B activation in myeloid, leukaemia, and epithelial cells. It also restricted NF-B activation brought on by tumour necrosis factor (TNF), interleukin-1 (IL-1), phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), and cigarette smoke. The sequential inhibition of IB kinase, IB phosphorylation, IB ubiquitination, p65 phosphorylation, nuclear translocation, acetylation, and NF-B-dependent reporter gene expression was linked with this activation. In acute myelogenous leukaemia patients' bone marrow-derived mononuclear cells, indole-3-carbinol inhibited constitutive NF-B activation, and this was associated with a reduction in cell proliferation. As a result, it can be said that indole-3-carbinol suppresses NF-B and NF-B-regulated transcription, and that this process may give the chemical the molecular foundation for its potential to reduce carcinogenesis. [17]

Additionally, For Parkinson's disease, the indole derivative fights oxidative stress neuroinflammation, as well as neurodegeneration. In vitro, indole derivatives reduced MPP+-induced cytotoxicity, decreased NO, IL-1, IL-6, and TNF-production, and inhibited MPP+-activated HMC3 cells' NLR family pyrin domain containing 3 (NLRP3) inflammasome activation. In vivo, MPTP-treated mice treated with NC009-1 had lessened motor impairments and non-motor depression, higher levels of dopamine and dopamine transporters in the striatum, and lessened oxidative stress as well as microglia and astrocyte reactivity. These protective effects were attained by upregulating SOD2, NRF2, and NQO1 and downregulating NLRP3, CASP1, iNOS, IL-1, IL-6, and TNF-. These findings support the idea that neuroinflammation and oxidative stress play a role in the pathogenic mechanism of Parkinson's disease and point to NC009-1 as a possible therapeutic candidate for the treatment of the disease [18].

4. Conclusion

The present study concludes that among several Schiff's base Indole derivatives, some of the compounds such as C1IN, C2IN, C6IN, C8IN and C9IN showed significant reduction in paw edema as well as inflammatory cytokines such as TNF-α and IL-1β. Hence, it can be concluded that the developed Schiff's base Indole derivatives such as can be alternative components as anti-inflammatory agents.

Acknowledgment

The authors would like to thanks Sunder Deep Pharmacy College, Dasna, Ghaziabad, Uttar Pradesh, 201009 and Amity Institute of Pharmacy, Noida, Uttar Pradesh, 201303 for providing the facilities to conduct the studies smoothly.

Conflict of interest

The authors declare no conflict of interest

Funding

Nil

References

1. Hoffman SB. Mechanisms of Antibiotic Resistance. Compend Contin Educ Pract Vet. 2001;23:464–473

2. Alshibl HM, Al-Abdullah ES, Haiba ME, Alkahtani HM, Awad GEA, Mahmoud AH, et al. Synthesis and evaluation of new coumarin derivatives as antioxidant, antimicrobial, and anti-inflammatory agents. Molecules. 2020;25(14):3251.

3. Zhang L, Bao M, Liu B, Zhao H, Zhang Y, Ji XY, et al. Effect of Andrographolide and Its Analogs on Bacterial Infection: A Review. Pharmacology. 2020.105(3-4):123-34.

4. Gaurav. GC–MS metabolomics and network pharmacology-based investigation of molecular mechanism of identified metabolites from Tinospora cordifolia (Willd.) miers for the treatment of kidney diseases. Pharmacogn Mag. 2022;18(79):548–58.

5. DeLong EF, Pace NR. Environmental Diversity of Bacteria and Archaea. Syst Biol. 2001;50(4):470-8.

6. Ceramella J, Iacopetta D, Catalano A, Cirillo F, Lappano R, Sinicropi MS. A Review on the Antimicrobial Activity of Schiff Bases: Data Collection and Recent Studies. Antibiotics. 2022.11(2):191.

7. Yadav S, Kumar N. Synthesis and Evaluation of Novel 4-Hydroxycoumarin Derivatives as Potential Anti-Microbial Agents. Orient J Chem. 2021;37(5):1132.

8. Jargalsaikhan BE, Ganbaatar N, Urtnasan M, Uranbileg N, Begzsuren D. Anti-inflammatory effect of polyherbal formulation (PHF) on carrageenan and lipopolysaccharide-induced acute inflammation in rats. Biomed Pharmacol J. 2019;12(4):1801–9.

9. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of lactobacillus on carrageenan-induced paw edema in male wistar rats. Int J Inflam. 2012;

10. Umar S, Zargan J, Umar K, Ahmad S, Kant C, Khan HA. Chemico-Biological Interactions Modulation of the oxidative stress and inflammatory cytokine response by

thymoquinone in the collagen induced arthritis in Wistar rats. Chem Biol Interact. 2012;197(1):40–6.

11. Maswadeh HM, Semreen MH, Naddaf AR. Anti-inflammatory activity of Achillea and Ruscus topical gel on carrageenan-induced paw edema in rats. Acta Pol Pharm - Drug Res. 2006;

12. Chaudhari SP, Baviskar DT. Anti-inflammatory activity of Xanthium indicum on carrageenan-induced paw edema in rats. Adv Tradit Med. 2021;

13. Odira HO, Mitema SO, Mapenay IM, Moriasi GA. Anti-inflammatory, Analgesic, and Cytotoxic Effects of The Phytexponent: A Polyherbal Formulation. J Evidence-Based Integr Med. 2022;

14. Bhat MA, Mohamed AAO, Mohammad R, Ansari MA, Abuelizz HA, Bakheit AH, et al. Indole derivatives as cyclooxygenase inhibitors: Synthesis, biological evaluation and docking studies. Molecules. 2018;23(6).

15. Ibrahim MM, Elsaman T, Al-Nour MY. Synthesis, Anti-Inflammatory Activity, and In Silico Study of Novel Diclofenac and Isatin Conjugates. Int J Med Chem. 2018;

16. Gaurav, Sharma I, Khan MU, Zahiruddin S, Basist P, Ahmad S. Multi-Mechanistic and Therapeutic Exploration of Nephroprotective Effect of Traditional Ayurvedic Polyherbal Formulation Using In Silico, In Vitro and In Vivo Approaches. Biomedicines. 2023;11(1).

17. Takada Y, Andreeff M, Aggarwal BB. Indole-3-carbinol suppresses NF-κB and IκBα kinase activation, causing inhibition of expression of NF-κB-regulated antiapoptotic and metastatic gene products and enhancement of apoptosis in myeloid and leukemia cells. Blood. 2005;

18. Chiu YJ, Lin CH, Lin CY, Yang PN, Lo YS, Chen YC, et al. Investigating Therapeutic Effects of Indole Derivatives Targeting Inflammation and Oxidative Stress in Neurotoxin-Induced Cell and Mouse Models of Parkinson's Disease. Int J Mol Sci. 2023;