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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY STUDY OF SCHIFF BASES COMPLEXES

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ABSTRACT : Schiff bases recently has many activities, they take interesting from many researchers. Two new Schiff bases were synthesized from 3-Phenyl-propenal (cinnamaldehyde) with amino acids (Tryptophan or Histidine) as ligands. Complexes synthesized from reaction of ligand with metal ion, copper sulphate were used for complexes preparation. The synthesized compounds identify by FT-IR and ^{1}HNMR spectra. Inflammation was induced by injection of fresh hen egg albumin in mice paw. Anti-inflammatory activity was estimated by measured thickness of mice paw, the complex that contain tryptophan show activity against inflammation compare with aspirin, which is used as standard drug. The activity may be attributed to tryptophan ring in the complex.

Key words : Anti-inflammatory, Schiff bases, tryptophan, histidine, complexes.

INTRODUCTION

Schiff bases are compounds containing azomethine group (-HC=N-). Hugo Schiff described that "They are condensation products of ketones or aldehydes with primary amines" in 1864 (Xavire and Srividhya, 2014). They have recently received considerable observation according to their good performance in coordination chemistry, unique anti-bacterial, anti-cancer and other physical activities (Cozzi, 2004). Schiff base complexes gained from amino acids are discovery applications in the understanding of many biochemical reactions. An amino acidis a kind of important biological substrate, which contains several N and O atoms. Cancerous cells have a much greater demand for amino acids than normal cells. Hence, amino acids Schiff bases may deliver an anti-cancer base to cancerous cells, thereby increasing the selectivity of anti-cancer cells (Antony *et al*, 2016). L-Tryptophan (Trp) is an essential amino acid, which is required for the biosynthesis of proteins. Also,it has an important role in nitrogen balance and the maintenance of muscle mass for body weight in humans (Zhang *et al*, 2009). In biochemical system histidine found in complexes coordinate with metals ions, that is a main biochemical character in many proteins.

Histidine found in many proteins by structural determination studies in x-ray of metalloproteins like carbonic anhydrase, carboxypeptidase, plastocyanin, or azurin among others have demonstrated. Moreover, in zinc metabolism the binding of histidine with zinc moiety in serum is necessary (Henkin, 1974).

Organic chemistry used Schiff bases widely, a large number of Schiff bases and their complexes were studied for their interesting and important properties. Schiff bases are compounds containing imine group (C=N) possessing a broad spectrum of biological activity (Al-Zoubi, 2013). The C=N bond is involved in several biological functions allowing the Schiff bases to behave, for illustration, as antimicrobial, anti-inflammatory, antitumor, or antiviral drugs (Aslam *et al*, 2012).

Inorganic elements play a central role in biochemical and biological medical processes, many organic compounds used in medicine do not only for the organic mode of action, some are activated or bio-transformed by metal ions metabolism.Incorporation of Schiff bases and metals in form of complexes showed some degrees of antibacterial and anti-inflammatory activity (Gupta *et al*, 1998; Yousif *et al*, 2018). The potential of these ligand-metal complexes as broad-spectrum antimicrobial agents, in-vitro will be verified a continuation of our researches in the field of bioorganic chemistry (Osowole *et al*, 2012a,b; Osowole *et al,* 2015). Schiff base derivatives complexation with several metals ions, showed capability

to forming mono-, di- or poly-nuclear complexes, they for that behaved as monodentate, bidentate or tridentate ligands depending on position and number of electron donating groups (Bukhari *et al*, 2005; Naz and Iqbal, 2011; Abdulghani and Hussain, 2015).

Inflammation is considered as a primary physiologic defense mechanism, this helps body to protect itself against infection, burn, toxic chemical, allergens, or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of chronic illness (Kshash, 2010; Al-Noor, 2013). Inflammation is response of tissue to injury, often injury cause by invading pathogen, it is characterization by increase blood flow to the tissue causing increased temperature, redness, swilling and pain (Nigam *et al*, 2009).

In this research, anti-inflammatory activity of Schiff base complex derived from cinnamaldehyde with tryptophan or histidine were studied. The anti-inflammatory action of these complexes were inspected by using egg albumin induced inflammation.

MATERIALS AND METHODS

Chemicals employed were analytical grade and used without further purification, melting points were determined in Stuart–SMP3 melting point apparatus. FTIR spectra were recorded by using FTIR 8400S SHIMADZU (Japan) Fourier Transform infrared spectrophotometer, the region used between (400-4000). The measurement done in Pharmaceutical Chemistry Department, College of Pharmacy, University of Basrah. ^{1}H-NMR spectra were recorded on Perkin Elmer 500 MHz instrument using tetramethylsilane (TMS) as an internal standard, DMSO.d6 were used as a solvent. ^{1}H-NMR measurement done at University of Tehran, Iran. The chemicals used in the preparations were supplied from the following companies: cinnamaldehyde (3-Phenyl-propenal)(Fluka), Solvents of absolute ethyl alcohol (Merck), diethylether, dichloroethane and metal salt $CuSO_4.6H_2O$ (B.D.H), tryptophan and histidine (Fluka).

Synthesis of Schiff bases

Ligands preparation (P1&P2)

The Schiff bases ligands were synthesized by first dissolving 1 mmol. liquid 3-Phenyl-propenal 0.132 gm in 20 ml of ethanol. This is followed by dissolving 1 mmol. of amino acid:0.204 gm, tryptophan in 20ml of ethanol. These two solutions were mixed together and stirred for 1 hr. This give proper media since the reaction is exothermic, Glacial acetic acid 5 drops was added to solution mixture, since acetic acid provides optimum pH for Schiff base reaction. The resulting mixture were then poured into a 250 ml reflux equipment, then heated undercontinuous refluxing for 12 hours by isomental electrical heater, the temperature maintained between 70-80^{o}C for condensation reaction to completely take place. The solid formed upon cooling was collected by filtration, washed three times with ethanol and recrystallized by hot ethanol. The purification of compounds were tested first by thin layer chromatography (T.L.C.) using different

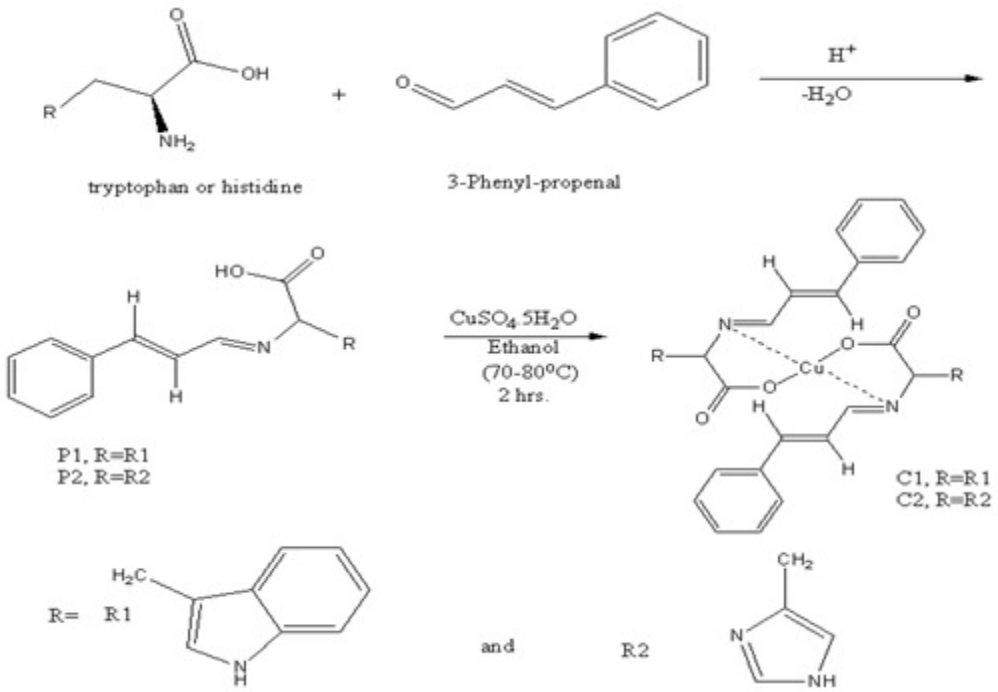

Fig. 1 : Schiff bases ligands and their complexes.

eluents. The product obtained was oven dried at 50ºC and weighed to give the desired brown crystal compound as the Schiff base ligand (P1) as illustrated in Fig. 1 (Hussain *et al*, 2014). P2 was synthesised by the same way by using 1 mmol. 0.115gm of histidine. All these reactions were monitored by TLC. The ligand was made by undergoing a complexation reaction with the metal ions, the complex was prepared in a ratio 2:1 ligand: metal ion to give a coordinated compound.

Synthesis of complexes (C1 & C2)

The ligands (P1 or P2) 2 mmol. dissolved in 20 ml of hot ethanol to give 2 molar solution. Next to the solution 2mmol. of metal salt (1 molar solution) was added, the metal salt solution was $CuSO_4.5H_2O$ dissolved in 20 ml ethanol 96% and 10 ml distilled water. Ligand metal salt mixture were heated with stirring by magnetic hotplate for 3 min. beforebeing reflux, the mixture then reflux for 3hrs at 80ºC, precipitate was formed after cooling, the precipitate was collected by filtration and dissolved in hot ethanol for first recrystallization, second recrystallization done by methanol to purify the complex product. The resulting crystals washed with 20 ml hot ethanol-methanol mixture in a 1:1 ratio. All these reactions were monitored by TLC (El-Nawawy *et al*, 2011).

Anti-inflammatory of Schiff base complexes

Swiss albino mice were divided into four groups of six animals each (n = 6), first group receive complex 1 and second group receive complex 2, the other two groups considered as positive and negative control. The potency of anti-inflammatory effect was determined through induction of inflammation by subplanter injection of fresh hen egg albumin (0.02 mL) in mice paw. The compounds were given to mice as a single dose by oral (75 mg/Kg), one hour before induced inflammation. The experimental carried out at 9.30 am to 1.50 pm. The paws of mice were measured by digital electronic micrometer device at zero time, after 60 and 120 min (Chakraborthy *et al*, 2012).

RESULTS AND DISCUSSION

Infrared Spectra of Schiff bases

The FTIR spectra of the synthesized Schiff bases P1 and P2 are recorded. The C=N stretch in metal complexes of cupric with Schiff basses C1 and C2 were found at 1701 and 1693 cm^{-1} compared with P1 and P2 were found at 1670 and 1635cm^{-1}, this band was shifted in the complexes spectra to lower wave numbers. Therefore, this shift in metal complexes suggest participation of azomethines C=N-M group in the nitrogen atom interacting with metal ions, thereby sharing in the coordination process(Chohan, 2006; Jesmin *et al*, 2014).

The disappearance of the absorption peaks at range 1710-1688 cm^{-1} may be attributed to the carbonyl group, which presence in the aldehyde compounds in the FTIR spectra indicating the complete participation in the condensation reactions.

3-(1H-indol-3-yl)-2-(((E)-3-phenylallylidene) amino) propanoic acid P1

Melting point m.p. 112-114ºC, yield: 87%, Brown powder, TLC, Ethanol: Dichloromethene, 4:6 retardation factor, Rf=0.80; FTIR (cm^{-1}): 1500(C=C), 1635 (C=N), 2920 (Aliph., C-H), 3028 (Ar-H), 4322 (-OH). ^{1}HNMR spectrum, ä, two signal appeared in the 2.46 due to d6 DMSO solvent and in 3.53 to HDO. 3.38 (t,1H,H-8), 3.25(d,2H,H-9),7.12 (t, 1H, H-3),7.17 (m, 4H, H-3',H-4', H-5'and H-6'),7.22 (d,2H, H-2, H-4), 7.27 (s,H,H-1'), 7.31(d,2H,H-1,H-5), 7.54 (d,1H, CH=N-), 7.63(s, H, -NH).

3-(1H-imidazol-4-yl)-2-(((E)-3-phenylallylidene) amino) propanoic acid P2

M.p.207-209ºC,umber needlescrystals, yield 75%, TLC, Ethanol: Toluene, 2.5:7.5, R_f=0.60; FTIR: 1589(C=C), 1618(C=N), 2927(Aliph., C-H), 3028 (Ar-H)3439 (-OH). ^{1}HNMR, 1.36 (t,1H,H-8), 1.29 (d,2H,H-9), 7.18(t,1H,H-3),7.28 (s, H, H-1'), 7.32(d,1H, 6), 6.41(d,1H,H-7), 7.35(d, H-2,H-4), 7.30(d,H-1,H-5), 7.54 (d, 1H, CH=N-), 9.66 (s, 1H, H-1',-NH), 7.45 (s,1H,H-1'), 7.07 (s,H-2').

Complex C1

M.p. 118-120ºC,reddish brown powder, yield 60%, Ethanol: Dichloromethene, 3:2, R_f=0.86; FTIR: 1497(C=C), 1654(C=N), 2839 (Aliph., C-H), 3059 (Ar-H). ^{1}HNMR,0.8 (t,1H,H-11), 1.18 (d,2H,H-12), 6.51(d,1H,H-7), 6.86 (d,1H,H-8), 7.21-7.33(m, Ar- H), 7.42 (s,1H, CH=N-), 9.62 (s, H, H-1') for N-H of the indole ring.

Complex C2

M.p. 135-137ºC, dark brown bowder,yield 79% Ethanol: Toluen,2:3, R_f = 0.79; FTIR: 1492(C=C), 1620(C=N), 2985(Aliph., C-H), 3097(Ar-H). ^{1}HNMR, 2.40(2H,d R_f,H-10),3.16(1H,t,H-9), 3.37(1H,t,H-10'), 6.88(1H,d,H-6), 6.62(1H,d,H-7), Ar-H 7.11(1H,d,H-3), 7.19(1H,d,H-3'),7.24(2H,t,H-2,H-4), 7.28(2H,t,H-2',H-4'), 7.31 (2H, d,H-1,H-5), 7.36 (2H,d,H-1',H-5'), 7.44(2H,s,H-11,H11'), 7.55(1H,s,H-12), 7.63(1H,s,H-12'), 7.83(1H,s,H-8), 7.88(1H,s,H-8'), 10.91(1H,s), 11.10(1H,s) for N-H of the imidazole ring.

The non-steroidal anti-inflammatory drugs inhibit peripheral pain, they inhibit enzyme types cyclooxygenase (COX), like COX-1 and COX-2, they for that obstructing

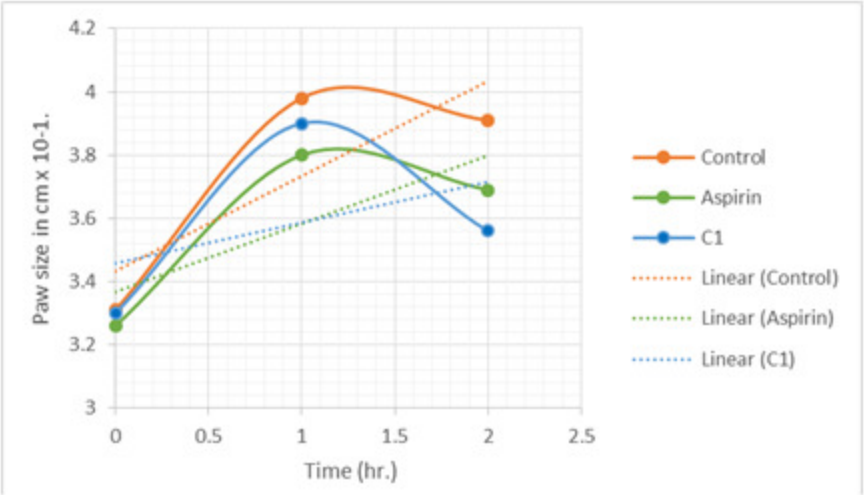

Fig. 2 : Anti-inflammatory of complex (1).

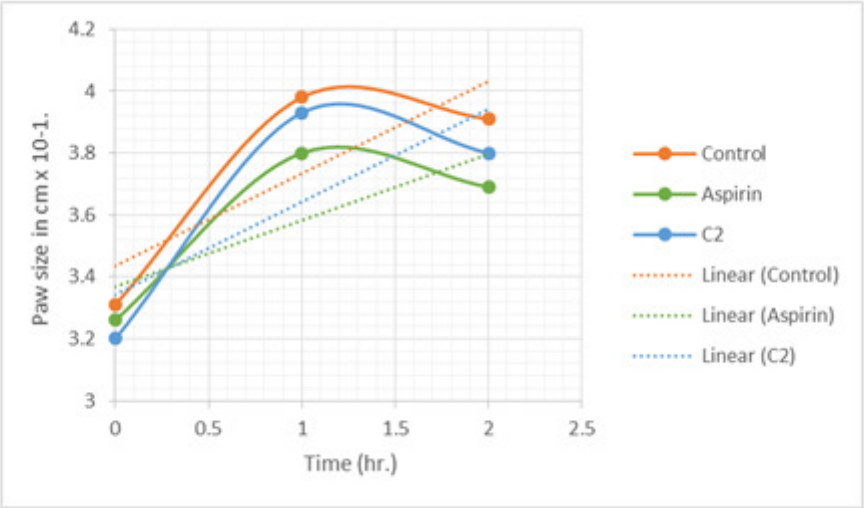

Fig. 3 : Anti-inflammatory of complex (2).

prostaglandins creation (Elisabetsky *et al*, 1995). The results of anti-inflammatory activity were shown in Table 1. The thicknesses of paw size at zero time were 3.3 and 3.2 mm. for treatment groups respectively. After 1 hr. of injection of egg albumin there are increasing in thicknessof paw size 3.90 and 3.93 mm., that indicate swelling and inflammation were happened. So after 2 hr. the two complexes C1 and C2 were shrunk thickness of paw size to 3.56 and 3.80 mm, respectively. The effect of complexes indicate reduced inflammation, the complex C1 was more active than complex C2, that because present of tryptophan instead of histidine as shown in Figs. 2 and 3. The mechanisms of prepared complexes may be inhibit peripheral pain similar to NSAID, but the central analgesic response of the compounds is not completely understood and may need further investigation (Demsie *et al*, 2019).

Table 1 : Anti-inflammatory activity of complexes.

Groups	The changed of paw size in cm × 10^{-1} at experimental period in hours(hr).		
	0-time	After 1 hr.	After 2 hr.
Control Negative control	3.31 ± 0.06 (0.2 ml water)	3.98 ± 0.13	3.91 ± 0.05
Aspirin Positive control	3.26 ± 0.04 (75 mg/kg)	3.80 ±0.11*	3.69 ± 0.07*
C1 (group 1)	3.30 ± 0.05 (75 mg/kg)	3.90 ± 0.07	3.56 ± 0.21*
C2 (group 2)	3.20 ± 0.07 (75 mg/kg)	3.93 ± 0.31	3.80 ± 0.08

CONCLUSION

The results of anti-inflammatory activity were taken by thickness of paw size. The effect of complexes indicate reduced inflammation, the complex C1 was more active than complex C2, that because present of tryptophan instead of histidine. The mechanisms of prepared complexes may be inhibiting peripheral pain similar to NSAID, but the central analgesic response of the compounds is not completely understood and may

need further investigation.

Conflict of interest : The authors declare that there is no conflict of interest.

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