Synthesis, Characterization and Staining Ability of Novel Azo Dye Based on Curcumin and Its Au(III) Complex

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Abstract: *Azo dye ligand (HMDA), namely* N-*(4-((*E*)-((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-yl)diazenyl)phenyl)acetamide was prepared by coupling diazonium salt of 4-aminoacetanilide with curcumin in basic conditions. The HMDA ligand and its Au(III) complex were characterized by elemental analysis, mass spectroscopy, ^{1}H-NMR, FTIR, UV-visible spectra, and molar conductivity. The molar ratio method was applied to ascertain the stoichiometric composition of the Au(III) complex in aqueous solution, which was 1:2 (metal ion to ligand). HMDA ligand binding with Au(III) ion used the enolate form moiety of curcumin under alkaline conditions which was observed by infrared spectra and investigated by elemental analysis and ^{1}H-NMR. HMDA and its Au(III) complex have been evaluated as stains for intestine, pancreas and kidney tissues of mice and exhibited important contrast. Both compounds showed the most potent staining activity toward blood cells, collagen, muscle fibers and cytoplasm in the selected tissues of mice. This azo dye and its complex of Au(III) succeeded in dyeing mice tissues, compared with the conventional stains.*

Keywords: *curcumin; azo dye; stain; coordination compound*

■ INTRODUCTION

Curcumin is a bright orange-yellow crystalline solid and it is widely used as a coloring and food additive. The light fastness for the dyed fabrics is improved by using azo curcumin dyes and their complexes compared to using only curcumin [1-2]. Azo curcumin derivatives with a variety of colors depending on the type of amine derivative investigated for natural fibers (cotton, silk, and wool) exhibited improving fastness properties of the dyed fabrics to washing and light fastness [1].

Bis-keto tautomeric form of curcumin dominates in acidic and neutral conditions as well as in the solid phase, which could act as a potential donor of hydrogen atoms, while the enolate form of curcumin under alkaline conditions predominates [3-5]. Due to its demonstrated antioxidant qualities, curcumin has recently attracted a lot of attention in the prevention and treatment of diseases. Turmeric contains curcumin, a hydrophobic, low-molecular-weight polyphenol that is frequently utilized in food [6]. Both forms of curcumin especially in the case of the enolate form (monobasic bidentate ligand) form stable complexes due to effective chelating agents that create stable complexes with the majority of known metal ions [7].

The curcumin probes have also been demonstrated to have better metrological characteristics when used with a nano-system, including low detection limits, repeatability, reproducibility, strong selectivity, and excellent storage stability [8]. Photophysical and inborn fluorescence characteristics are present in curcumin. Binding to metals changes its luminous characteristics. As a result, it is used to improve the efficiency of nano-sensors for detecting important chemicals, ions, metals, and contaminants that are harmful to the environment

as well as, as a natural fluorophore and electrochemical transducer. Additionally, it is employed to chelate metal ions, and the creation of functionalized nanoparticles has made use of this characteristic [9].

Curcumin is considered an excellent scavenger of most reactive oxygen species (ROS). The phenoxyl radicals can be easily formed by abstractable the hydrogen from the phenol-OH group of curcumin to form a stable keto-enol structure by the resonance [2,10].

The staining process is important because it makes things more visible by sharpening the contrast between the organism and its surroundings. It makes it possible to distinguish between a variety of morphological types, such as shape, size, and arrangement, as well as to ascertain an organism's staining characteristics, make a potential diagnosis of a direct illness, and demonstrate a pure culture [11]. The current study is concentrated on the synthesis, structural, and application aspects of the monoazo ligand produced from diazotizing of 4-aminoacetanilide and the active methylene coupling component of curcumin and its Au(III) metal complex and to evaluate the efficacy of azo dye and its Au(III) complex in staining mice tissues (pancreas, intestine, and kidney tissues) comparing with the conventional stains.

■ EXPERIMENTAL SECTION

Materials

4-Aminoacetanilide was purchased from Merck (purity 96%). Sodium hydroxide (NaOH, 98%) was prepared from Thomas baker. Curcumin crystalline was supplied from CDH (purity 96%). Hydrochloric acid (HCl, 38%) was purchased from CGH. Dimethyl sulfoxide (DMSO, 99%) was purchased from LOBA. Hydrogentetrachloroaurate(III) trihydrate was brought from Glentham Life Science (purity 99%).

Instrumentation

The infrared spectra were recorded by Shimadzu FTIR 8400S spectrophotometer in the range of 400–4000 cm^{-1} by using potassium bromide pellet, UV-visible spectrophotometer (UV-1650 PC) was used to record UV–vis spectra using quartz cuvettes 10.0 mm in diameter in the region of 200–1100 nm. Melting points were recorded by using Sturat digital melting Point/SMP3. NMR spectra were done by Bruker 500 MHz. Mass spectra were done by using Agilent 5375 USA. Elemental analysis (C.H.N) was done by using Elemental analyzer-EA-300.

Procedure

Preparation of the HMDA dye

This dye was prepared by stirring for 2 h in two steps. The first step is to prepare the diazonium salt, which was prepared by dissolving 0.3 g (0.002 mol) of 4-aminoacetanilide in 15 mL ethanol, then adding 3 mL of HCl 12 M and cooling the solution to 0 °C. Sodium nitrite (0.2 g, 0.002 mol) was dissolved in 5 mL of distilled water under cooling to 0 °C, which was added to the acidic solution of 4-aminoacetanilide to produce the diazonium salt. The solution of the diazonium salt was left for 1 h under cooling and stirring then the solution was mixed with the cooling solution of curcumin (0.5 g, 0.002 mol) in 10% NaOH (5%). The mixture solution was left overnight, then it was filtered and washed with distilled water many times then the powder was put in a desiccator to dry. Elemental analysis of the ligand Experimental (%), C: 66.57, H: 5.01, N: 7.91, Theoretical (%), C: 66.78, H: 5.14, N: 7.94.

Au(III) complex preparation

The HMDA ligand (0.24 g, 0.00046 mol) was dissolved in 20 mL of ethanol. Then it was mixed with NaOH (0.02 g, 0.00046 mol). The ligand solution was added slowly to the $HAuCl_4$ (0.08 g, 0.00023 mol), dissolving in 5 mL distilled water. The reaction mixture was refluxed for 1 h. The reaction solution was left overnight at room temperature, then was filtered, washed with a little cold water, and dried. Elemental analysis of the Au(III) complex, Experimental (%), C: 53.95, H: 4.01, N: 6.51, Theoretical (%), C: 54.02, H: 4.06, N: 6.52.

Histological staining process

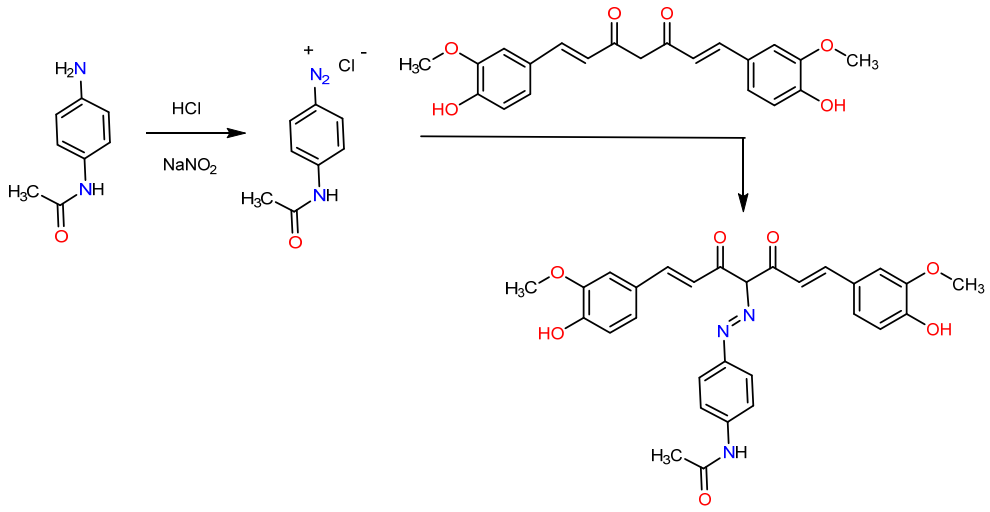
Slides containing wax-embedded small intestinal, pancreas, and kidney tissues were dewaxed in 100% xylene for 3 × 10 min each. The tissue was placed twice in 100% ethanol for 10 min. Subsequently, they were placed in 70% ethanol for 2 × 5 min. Slides were then rehydrated twice in distilled water for 5 min each. Slides

were immersed in bouins solution for 1 h at 59 °C then washed and stained by hametoxyline and washed by distal water, used HMDA ligand and Au(III) complex were used as a counter stain for 20 min at room temperature. Subsequently, slides were rehydrated by grading alcohol. Finally, slides were mounted in DPX. Also, the counter stain by routine H&E process sections was visualized using an epifluorescence microscope, and images were captured with a Canon digital camera (DS126371, Canon Inc, Japan).

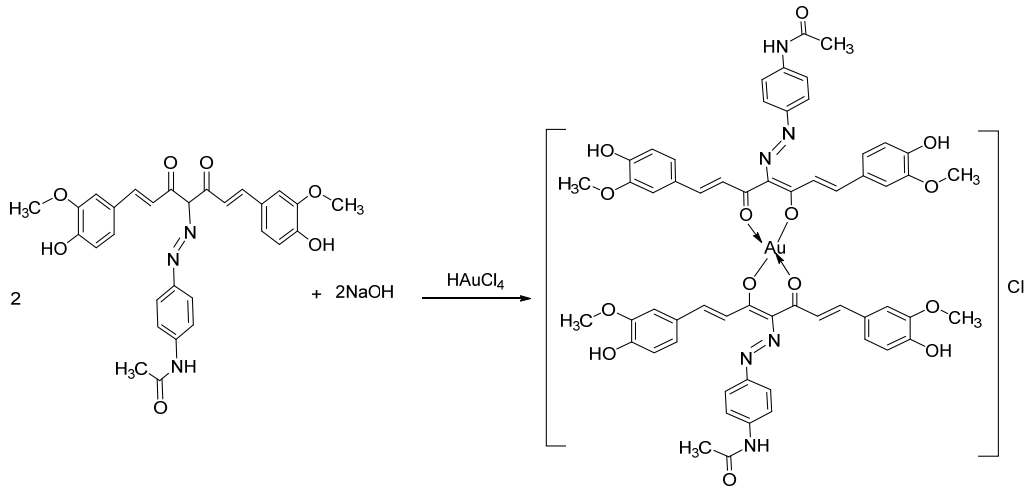
■ RESULTS AND DISCUSSION

The azo curcumin ligand was prepared by reacting diazonium salt of 4-aminoacetanilide with curcumin, as shown in Scheme 1. The resulting dye, namely *N*-(4-((*E*)-((1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-yl)diazenyl)phenyl)acetamide (HMDA) was obtained as a light brown powder with a percentage of 82% and melting point 188 °C. The solid HMDA ligand was insoluble in water but completely soluble in some organic solvents such as dimethylsulfoxide, ethanol, and acetone.

The Au(III) complex was prepared by reacting the $HAuCl_4$ with two equivalents of HMDA ligand, as shown in Scheme 2. The mass spectrum of HMDA dye (Fig. 1) provided a molecular ion peak at m/z = 532, and the molecular ion peak of the Au(III) complex was at m/z = 1254, which corresponds to predict the molecular



Scheme 1. Preparation steps of HMDA ligand of azo dye based on curcumin



Scheme 2. Preparation of Au(III) complex for HMDA ligand of azo dye based on curcumin

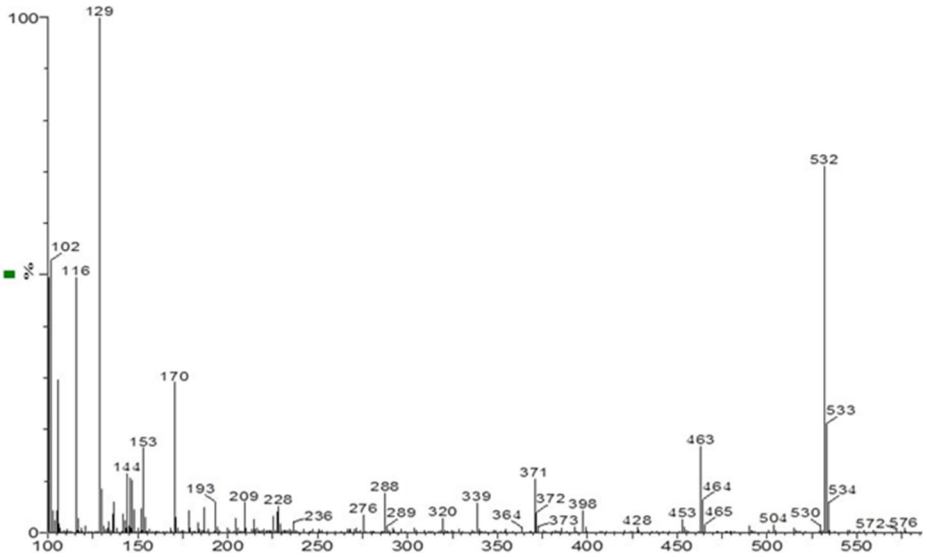


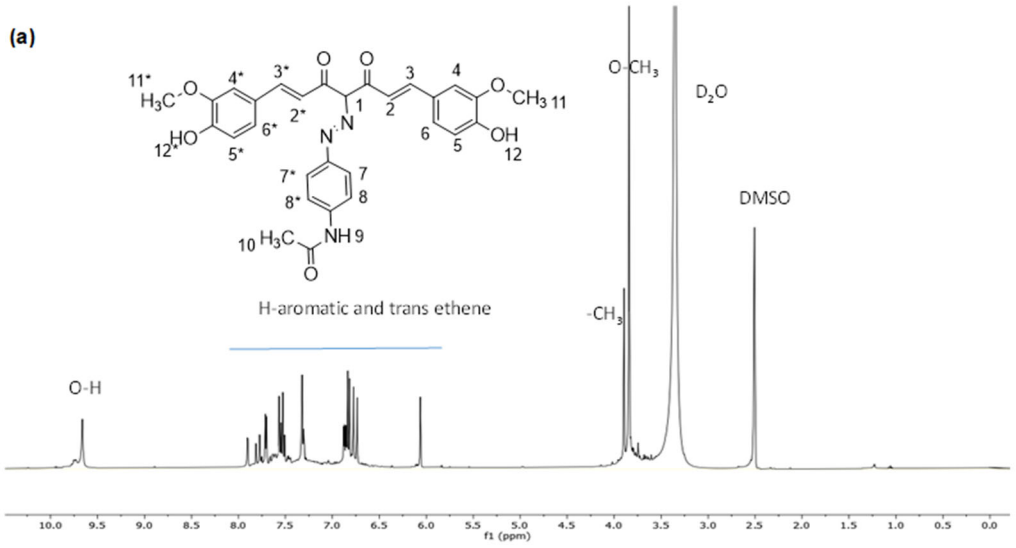
Fig 1. Mass spectrum of HMDA ligand of azo dye based on curcumin

weight of HMDA ligand and the $[Au(HMDA)_2]Cl$ formula of Au(III) complex.

The reaction of Au(III) with HMDA ligand in ethanol under reflux led to high yield Au(III) complex, and the results of the elemental analyses of the synthesized Au(III) complex were in good accord with what the proposed formula demanded.

^{1}H–NMR spectrum of HMDA ligand as shown in Fig. 2, exhibited many signals related to O–H and N–H at 9.62 and 7.91 ppm, respectively [12]. The signals exhibited at 3.91 and 3.82 ppm due to $O{=}CCH_3$ and $O{-}CH_3$, respectively. The other protons exhibited in the range of 6.55–7.91 ppm. The chemical shifts of the HMDA ligand are 9.62 (2H, s), 7.91 (1H, d), 7.79 (2H, d), 7.71 (2H, d), 7.55 (2H, d), 7.52 (2H, d), 7.33 (2H, d), 6.87 (2H, d), 6.76 (2H, d), 6.55 (1H, s), 3.91 (6H, s), and 3.82 (6H, s).

^{1}H-NMR spectrum of the Au(III) complex was compared to the free ligand of curcumin to gain more information about the nature of the metal complex in the solution. Upon complexation with Au(III), the singlet signal in position 1 in the free ligand vanished in the spectrum of complex. This proton was removed by using an equivalent NaOH base. The aromatic protons and ethene units exhibited in the range of 6.2–7.6 ppm. The signal at 9.85 ppm in the spectrum of the complex is



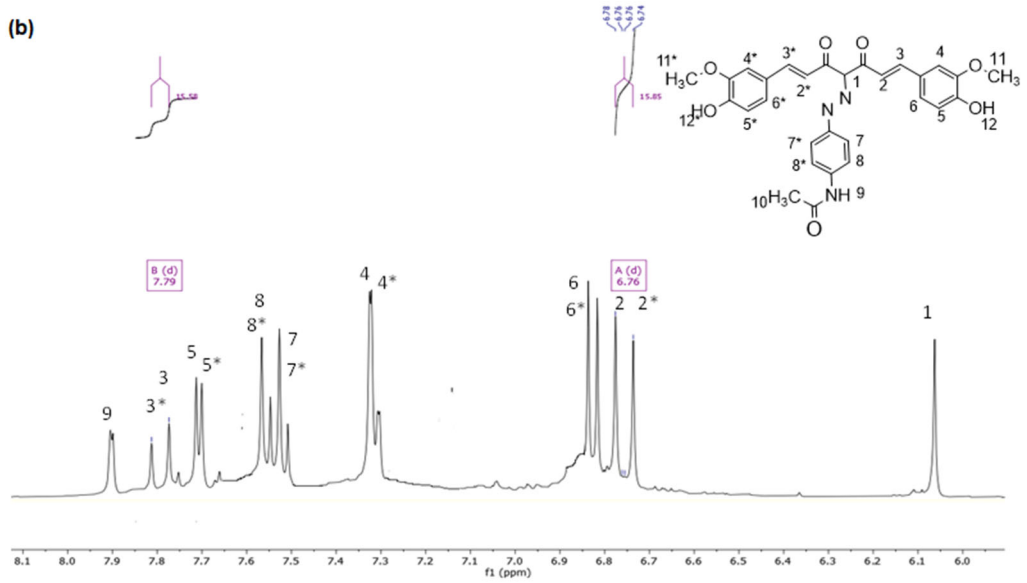


Fig 2. (a) ^{1}H-NMR spectrum of HMDA ligand of azo dye based on curcumin in DMSO-d_6 solvent and (b) the aromatic region of ^{1}H-NMR spectrum of HMDA ligand

due to –OH groups. The signal at 3.4 ppm is due to –CH. The signals exhibited at 3.74 and 3.53 ppm due to O=C–CH_3 and O–CH_3, respectively.

IR Spectra

The important function groups of the HMDA ligand and its Au(III) complex were investigated by infrared spectroscopy. The IR spectra of the HMDA ligand and its Au(III) complex were done by using a KBr disc. Their significant bands are summarized in Table 1, and Fig. 3 and 4 represent their IR spectra.

The IR spectrum of azo ligand (HMDA) revealed the band's absence of the –NH_2 stretching bond of acetanilide and the appearance of N=N stretching at 1415 cm^{-1}, indicating the diazotization of 4-aminoacetanilide and formation of the azo dye of curcumin [13]. The spectrum of HMDA ligand shows a medium band at 3360 cm^{-1} due to N–H group of acetanilide [14] and a medium band at 3418 cm^{-1} due to O–H groups of curcumin. A band had been exhibited at 3060 cm^{-1} in the HMDA ligand spectrum that is due to vibration of C–H of phenol ring and other bands at 2960 and 2837 cm^{-1} are due to the aliphatic stretching of C–H in curcumin. These bands of HMDA ligand showed in the same position in the spectrum of Au(III) complex [15-16].

A band at 1669 cm^{-1} in the HMDA spectrum is due to C=O group of curcumin, which shifted to low frequency

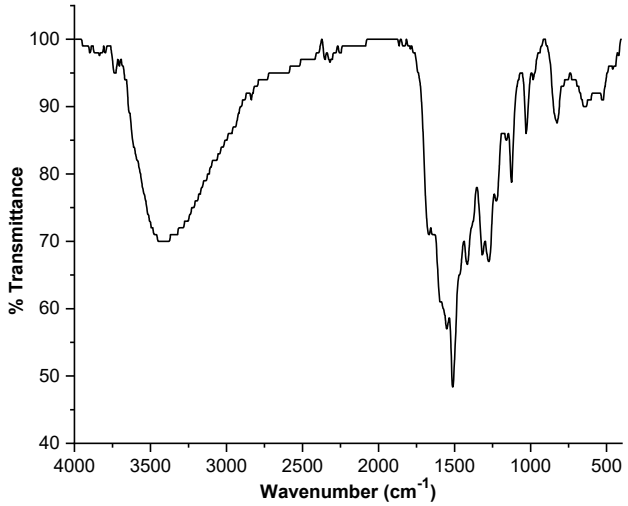


Fig 3. The IR spectrum of HMDA ligand

Table 1. The important vibrations of HMDA ligand and its Au(III) complex

Compound	–OH	–NH	–CH aromatic	–CH aliphatic	C=O	C=O curcumin	C=C	N=N	M–O
HMDA ligand	3418	3360	3060	2960, 2837	1672	1669	1510	1415	-
Au(III) complex	3396	3304	3072	2954, 2837	1670	1597	1513	1426	523

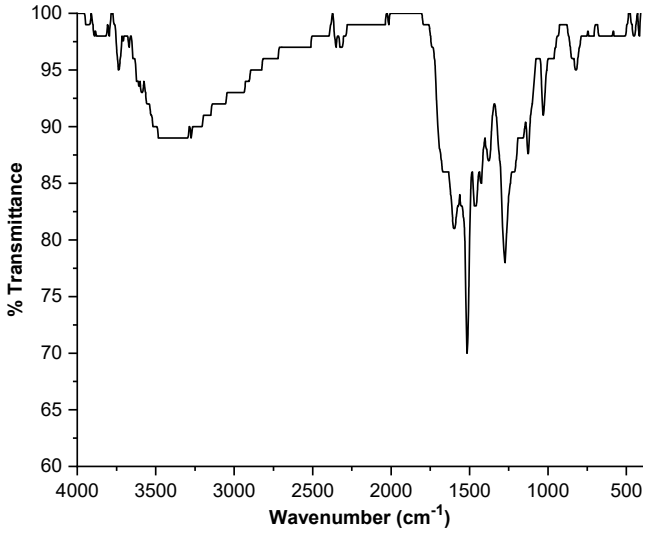


Fig 4. The IR spectrum of Au(III) complex for HMDA ligand

in the spectrum of the gold complex [17]. That indicates that the oxygen of the C=O group is involved in the coordination with metal ions of the Au(III) complex. The IR spectrum of the Au(III) complex appeared to have a new absorption band at 523 cm^{-1}, which is attributed to (M–O) [18]. The IR spectral data indicate that HMDA ligand behaves as a bidentate chelating ligand coordinating through the positions of oxygen of keto-enol groups which were in the resonance process [6].

UV–vis Spectra of HMDA Ligand and Au(III) complex

Electronic transition measurements were conducted in dimethylsulfoxide at room temperature. UV-vis spectra of HMDA ligand and Au(III) complex are listed in Fig. 5. The HMDA ligand showed bands at 260 and 429 nm. These bands are due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively [19-22]. The Au(III) complex spectrum exhibited bands at 386 and 513 nm due to $^1A_{1g} \rightarrow {}^1E_g$ and $^1A_{1g} \rightarrow {}^1A_{2g}$ transitions, respectively. These bands represent pretty accurately the square planar shape of the Au(III) ion, and the band at 263 nm is an electronic transition due to the ligand [23].

Staining Process

The tissues of mice stained by yellow color in aqueous solutions under effects of HMDA ligand and Au(III) complex. We added the solution of bouins as a mordant of the staining, and we used the procedure of staining as in reference [24-25]. The effect of portions of HMDA ligand and Au(III) complex on tissue sections as a counter stain for the hematoxylin showing staining ability even after applying them for 20 min at room temperature stain which showed collagen fiber, red blood cells, cytoplasm deep yellow color in HMDA ligand and Au(III) on intestine tissues of the mice, while the stain

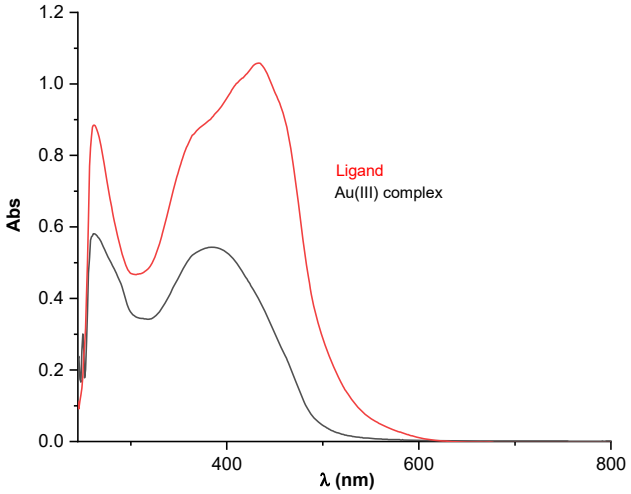


Fig 5. UV-visible spectra of HMDA ligand and Au(III) square complex

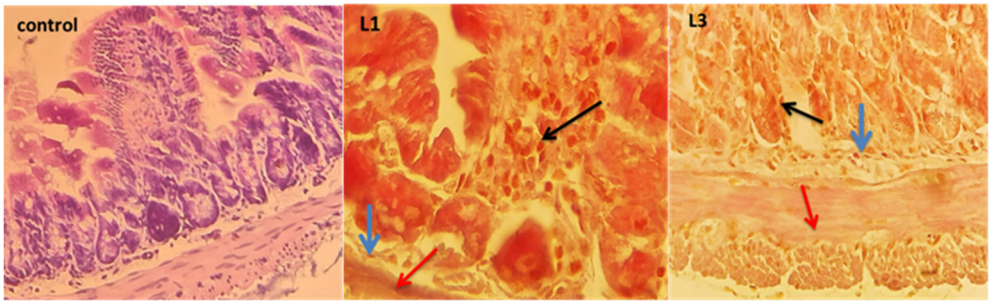


Fig 6. Microscopic images for mice intestine tissue HMDA ligand (L1), Au(III) complex (L3) stain RBC (black arrows), collagen fiber (blue arrows), muscle fiber (red arrows), and control stains of hematoxylin and eosin technique (X100)

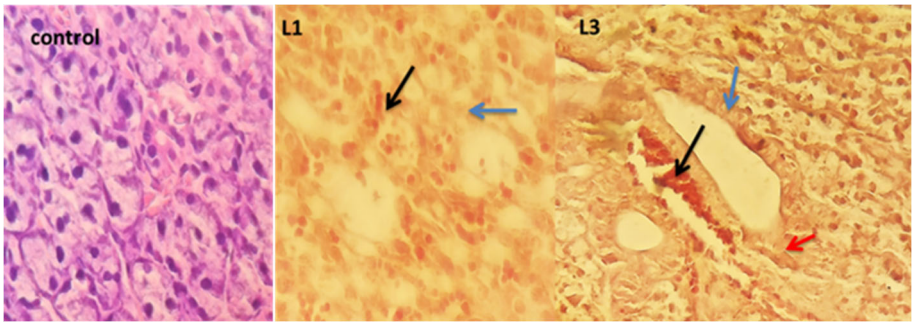


Fig 7. Microscopic images for mice pancreas tissue HMDA ligand (L1), Au(III) complex (L3) stain RBC (black arrows), collagen fiber(blue arrows), and control stains of hematoxylin and eosin technique (X200)

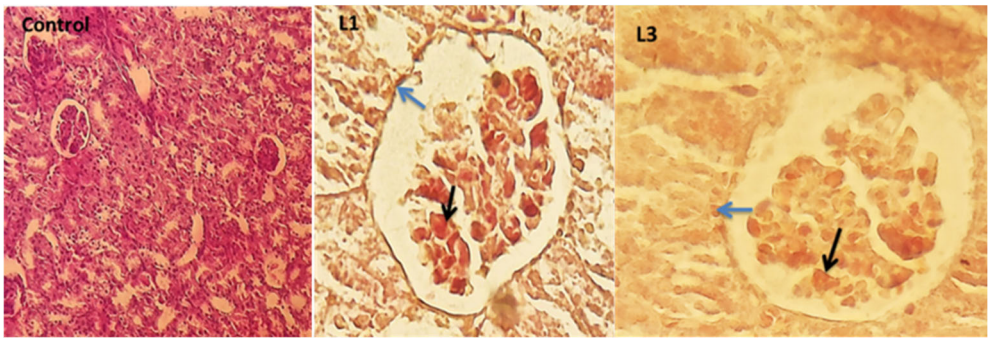


Fig 8. Microscopic images for mice kidney tissue HMDA ligand and Au(III) complex stain RBC (black arrows), collagen fiber (blue arrows), and stains control of hematoxylin and eosin technique (X100, X200)

gave the same level at the HMDA ligand and Au(III) complex in mice pancreas and kidney sections (Fig. 6, 7, and 8). In the case of control, we used stains of hematoxylin-eosin where the nuclei were stained blue, and cytoplasm with collagen fiber and red blood cells were stained pink (Fig. 6 ,7, 8, and control).

We noticed that the general staining HMDA ligand and Au(III) complex improved the staining technique for RBC cells and collagen fiber in the intestine tissues of mice accompanied with moderate stain in the case of Au(III) complex (Fig. 6). In Fig. 7 and 8, the general staining by HMDA ligand and Au(III) complex improved the staining technique for RBC cells and collagen fiber in pancreas and kidney tissues of mice.

■ CONCLUSION

In the present work, new azo dye and its Au(III) complex containing curcumin were successfully synthesized and characterized. The HMDA ligand is bidentate and Au(III) complex is a square planar. The mole ratio of reacting the ligand to metal is 2:1. It is noteworthy to conclude that azo dye and its Au(III) complex could be a safer alternative stain for the natural stains where our dyes have shown similar staining characteristics as compared to hematoxylin and eosin dyes. Dyes have the ability to stain nuclear and cytoplasmic in mice tissues of the pancreas, kidney, and intestine tissues. Azo stain showed superior staining properties in mice tissues such as collagen, muscle fibers and red blood cells, which showed better contrast and therefore found application in collagen and muscular disorders.

■ AUTHOR CONTRIBUTIONS

The authors have the same level of contribution in all parts work. All authors agreed to the final version of this manuscript.

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