ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at : www.jgpt.co.in

RESEARCH ARTICLE

Studies on Anti-Inflammatory, Antioxidant and Free Radical Scavenging of Cu (Ii)-N-Acetyl-Para-Aminophenol (APAP) Based Complex

Ouiza Hamrani[1*], Mahmoud El Amine Boudoua[2], Sultana Boutamine[1], Safia Kellou-Tairi[3], Zakia Hank[1]

1. *USTHB, Laboratoire d'Electrochimie-Corrosion, Métallurgie et Chimie Minérale, Faculté de Chimie, BP 32 El Alia 16111 Bab-Ezzouar, Alger, Algérie.*
2. *Institut Pasteur, Route du Petit Staouéli, Dély-Ibrahim 16302, Alger, Algérie.*
3. *USTHB, Laboratoire de Physico-Chimie Théorique et de Chimie Informatique, Faculté de Chimie, BP 32 El Alia 16111 Bab-Ezzouar, Alger, Algérie.*

***Corresponding Author: Ouiza Hamrani**

Abstract

N-acetyl-para-aminophenol (APAP) or paracetamol–copper based complex with [Cu(APAP) $_2$(OH)$_2$.H$_2$O] formula and noted Cu-APAP has been synthesized and characterized on the basis of elemental analysis, conductivity, IR and thermal (TG/DTG), NMR and electronic spectral studies. The value of molar conductance of the complex indicates a non-electrolyte nature. The antimicrobial activity of the complex was evaluated against several pathogenic microorganisms to assess its potential. The copper complex was found to be more active against Gram-positive than Gram-negative bacteria. The pharmacological and toxicological ability of the complex were equally explored. The results are compared to those obtained with the unbound drug molecule. The metal complex has been tested for antioxidant and catalase-like activity in order to assess its catalytic properties. The results are encouraging.

Keywords: *N-acetyl-para-aminophenol (APAP), Cu (II), Pharmacology, Toxicology and anti-microbiology.*

Introduction

The study on complexation of drug molecules with various metal ions is an important field of research in the chemical, biochemical, medicinal and pharmacological point of views [1]. Our body possesses a large number of metal ions for operating normal physiological activities and we also intake a number of metals as drug, dietary factor, drinks and we also come in close contact with different drugs. It's also well established that many pathological situations involve deregulation in the metabolism of metals: therapeutic responses are then necessary and although most drugs or compounds used in medicine are purely organic.

The challenge is to enhance the properties of these drugs by complexing them and to study their interaction with the trace elements present in the human organism. In fact, the complexation offers the metal ion a multitude of coordination possibilities and a wide range of geometries.

As a result, the metal complexes, due to their thermodynamic and kinetic properties, and in some cases their redox activities, offer novel mechanisms of action that organic compounds do not exhibit themselves so it's very important to control all these properties to obtain the desired therapeutic effect when a drug or a metal complex is introduced into the body or the cell [2-4]. The interest of this study is to examine the modifications that the metal can make to the properties of an organic molecule when the latter is coordinated to it.

Copper complexes have attracted great deal of attention due to their therapeutic applications as antimicrobial and antioxidant so; we have been interested in the study of

the complexation of this metal by some drug molecules such as N-acetyl-para-aminophenol (APAP) or paracetamol. Some works report similar study [5-9] but no study relating to its biological capacities was carried out. Copper is an element redox which is essential to the human organism exceptionally for the correct function of the brain. It is an essential element of the cytochrome c oxidase and certain enzymes in which it is associated with other elements as it is the case in the superoxyde dismutase (SOD, Cu/Zn)). Moreover, copper is a cofactor necessary for many specific enzymes as those which order homeostasis.

Experimental

Materials and Methods

All chemicals used were analytical reagent grade products without further purification. Copper (II), was used as chlorure salt and were purchased from Fluka. Paracetamol $C_8H_9NO_2$ was provided from Algerian Pharmaceutical Industrial Group (SAIDAL).

Synthesis of the Complex (Cu-APAP)

The complex was prepared by mixing twice amount of paracetamol 18 mmol (2.722 g) and 9 mmol (2.180 g) of $CuCl_2{\cdot}6H_2O$ in MeOH/H_2O (50/50, w/w) solvent. The reaction medium pH is adjusted to pH = 9, using a concentrated ammonia solution. The mixture was then refluxed under backward flow at temperature maintained lower 60°C for 6 h and left to stand overnight. The precipitated complex was filtered, washed with distilled water followed by water- ethanol mixture (50:50v proportion). The complex thus prepared, was dried with the drying oven during a few days at 60°C.

Spectral and Physical Measurements

Elemental analyses (C, H, N and M) were performed at the Central Service of Analysis, CNRS (Solaize-Lyon, France). Infrared spectra (4000-400 cm^{-1}) were recorded as KBr pellets on FTIR Nicolet Avatar 330 spectrophotometer. UV-vis absorption spectra were obtained in solid state or in DMSO solution from UV-Jasco V-650 spectrophotometer and recorded in 190-900 nm range.

^{1}H and ^{13}C NMR measurements were recorded with a RMN Bruker AM 300 spectrometer. Chemical shifts are expressed in ppm downfield from TMS.

Thermo gravimetric measurements (room temperature-600 °C) were recorded on a TA instrumentQ500 thermo gravimetric analyzer at a heating rate of 10 °C/min.

Theoretical Calculations

Theoretical calculations for the paracetamol and its Cu (II) complex were performed to optimize the geometry and to obtain the spectral features using Jaguar program included in the Schrodinger package for Linux platform, with the aid of Maestro GUI Schrödinger [10]. 3D molecular modeling of the ligand and the complex was carried out using density functional theory (DFT) at the B3LYP level for the ligand and UB3LYP for the Cu(II) complex, using Lacvp** as basis set [11].

Antimicrobial Activity (in vitro)

The antimicrobial activity of ligand and complex were tested against standard strains of Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* CIP 53-160) and one yeast (*Candida albicans* ATCC 10231) using the agar-disc diffusion method.

Mueller–Hinton agar (MHA) [12] and Sabouraud dextrose agar (SDA) were used to test the sensitivity of the bacteria and the yeast. The MHA and SDA were distributed into sterile Petri dishes. The bacteria were incubated in nutrient agar Mueller Hinton (MH) at 37 °C for 24 h. The yeast cultures were grown on Sabouraud dextrose broth (SAB) at 28 °C for 48 h [13, 14].

The cultures of the bacteria and yeast were injected into the Petri dishes (9 cm) in the amount of 50 µL. The ligand and the complex were dissolved at a concentration of 1.25 mg/mL in DMSO. The diameters of inhibition zones were measured in millimeters (mm). To ensure that the solvent had no effect on bacterial growth, a control test was performed with a test medium supplemented with ethanol and copper chloride salt following the same procedures as used in the experiments. Ampicilline and Amoxicilline were used as a standard reference in the case of bacteria while, Amphotericine B was used as a standard antifungal.

Toxicity Study

Experimental Animals

Mice of NMRI strain (17–22 g) of both male and female sex in-house bred at the Animal House of Institute Pasteur, Algiers, Algeria were used in this experiment.

Mice were housed in polypropylene cages lined (Length 55 cm, width 33 cm, height 19 cm) with husk in standard environmental conditions (temperature 22 ± 3°C, relative humidity 30-70 and 12:12 light: dark cycle). The animals are acclimatized to the conditions of the animalery of the laboratory of quality control of the IPA during about fifteen days before the experimentation. They were fed standard pellet diet (mice feed provided by ONAB Algiers, Algeria) ad libitum and access to water. Oral acute toxicity of complex was performed according to OECD guidelines [15] and respecting national rules on animal care.

Acute and Subacute Toxicity Study

The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided into two groups. Group A was kept as control and Group B received metal complex, control group received only water. The animals were observed for gross behavioral, neurologic, autonomic and toxic effect of time for 24 h and then daily for 14 days. The acute toxicity is expressed as an LD50 value. The toxicological effect was assessed on the basis of mortality.

Anti-inflammatory Assay

Carrageenan induced paw oedema model was used to determine the anti-inflammatory activity of the complex by the method of Winter and al. [16] Paw edema was induced by injecting 0.1 mL of 2% of Carrageenan in physiological saline solution into the sub plantar tissues of the left hind paw of each mouse [17]. Twenty Albino Swiss mice were allowed to fast for 18 hours and divided into four groups of five animals each. First batch (negative control) was treated with 0.5 mL of normal saline solution.

Second batch (positive control) received orally, 50 mg/Kg-body weight of diclofenac. Third and fourth batch received orally, copper complex (200.1 and 400.2 mg/Kg-body weight), respectively. The animals were sacrificed 4 hours after Carrageenan injection by ether inhalation. The hind legs are removed and weighed. Inhibitory activity was calculated using the following formula:

Percentage inhibition = 100 x [1- (L – R) / (Lc – Rc)]]

R: main of weight of right hind paw of mice treated with standard or studied compound

L: main of weight of left hind paw of mice treated with standard or studied compound

R_C: main of weight of right hind paw of mice of batch treated with physiological saline solution

Lc: main of weight of left hind paw of mice batch treated with physiological saline solution

Antioxidant Assay

The free radical scavenging effects of APAP and Cu-APAP complex with DPPH radical were evaluated with various concentrations (1, 2.5, 5, 10, 25, 50, 100, 150, 200, 250, 500, and 1000 μg/mL). The sample of the test compound (3 mL) was mixed with 1 mL of methanolic solution containing DPPH radicals. The mixture was shaken vigorously and left in the dark. After 30 min incubation period at room temperature, the reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discoloration using the equation: % Scavenging effect = $[(A_0 – A)]/A_0] \times 100$, where A is the absorbance of the solution when the tested compound has been added and A_0 is the absorbance of the DPPH solution [18]. Results are compared to those obtained when ascorbic acid (vitamin C) is used as a standard.

Catalase-like Activity

The study of the catalase-like activity (disproportionation reaction of H_2O_2 into H_2O and O_2) was carried out by volumetric determination of the oxygen evolved with a gas-measuring burette (with a precision of 0.1 mL). All reactions were carried out in a 250 cm^3 reactor containing a stirring bar under air. Distilled water (20 cm^3) was added to the complex and the flask was closed with a rubber septum. Hydrogen peroxide (2 mL at 83.3 N) was injected through the septum with a syringe at pH 5-6. The reactor was connected to a graduated burette filled with

water, and dioxygen evolution was measured volumetrically.

Statistical Analysis

Within studied groups, comparisons were performed. Student's test was used to assess the significance of differences seen between groups of animals. The results were considered significant at $p < 0.05$.

Results and Discussion

Characterization and Theoretical Calculations

Characterization

The distorted square planar complex [Cu (II) (APAP) $_2$(OH) $_2$].H_2O was carefully characterized by elemental analyses. $C_{16}H_{22}CuN_2O_7$ (F.W., calc. 417.99; found 416), yield: 70.86%. Anal. Calcd.: C, 48.00; N, 7.00; H, 4.40; Cu, 15.91%. Found: C, 49.01; N, 7.55; H, 4.12; Cu, 15.98%.

FTIR Study

The IR spectral data (Table 1) suggested that APAP (paracetamol) behaves as a monodentate coordinated ligand to the metal ions via the phenolic oxygen.

Table 1: IR data

Peak (cm^{-1})	v(OH)	v(NH)	v(CH_3)	δ(NH)	v(C=O)	v(C=C-H)	v(C-N)	δ(CH_3)
APAP	3200-3100	3326	2800-2700	1611	1666	1561 1513 1444	1281 1244	1376_{asym} 1328_{sym}
Complex	3230 intermol.	3327	2800-2700	1593	1656	1560 1510 1440	1279 1241	1370_{asym} 1320_{sym}

NMR Study

APAP (paracetamol) was identified within the complex par NMR (^{1}H and ^{13}C) investigation; this study is illustrated by Tables 2 and 3.

Table 2 : *^{1}H* NMR data

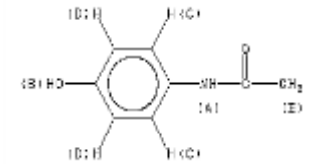
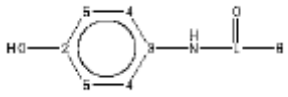
APAP structure	APAP in CD_3OD/δ (ppm)		Complex in CD_3OD/δ (ppm)	
	A	9.66 (1H)	A	9.95 (1 H)
	B	9.14 (1H)	B	9.22 (1 H)
	C	7.35 (2 H)	C	7.37 (2 H)
	D	6.69 (2 H)	D	6.72 (2 H)
	E	1.99 (3 H)	E	1.98 (3 H)

Table 3 : *^{13}C* NMR data

APAP structure	APAP in DMSO/δ (ppm)		Complex in DMSO/δ (ppm	
	1	171.38	1	167.44
	2	155.39	2	153.15
	3	131.69	3	130.99
	4	123.38	4	120.91
	5	116.20	5	114.97
	6	23.51	6	23.61

Spectroscopic Study

The electronic spectrum of Cu-APAP complex displays three bands. The former was sharp at 45454 cm^{-1} while the second as a shoulder centered at 33333 cm^{-1}. The two bands are assigned, respectively to π→π* of phenolic group and n→π* transitions of carbonyl group. The last band is broad and centered around 20000 cm^{-1}. It corresponds to the overlapping of $^2B_{2g} \rightarrow {}^2E_{1g}$ and $^2B_{2g} \rightarrow {}^2A_{1g}$ d-d transitions of Cu (II) ion in a square planar geometry with a CuO_4 environment [19-24].

Thermal Analysis

The TG curve indicates a significant stability of the complex up to T= 120 °C.

Above this temperature, the complex starts to decompose and the thermo gram presents three main distinct stages which are maximized at 210, 300, and 400 °C on the DTG curve. According to the hypothesis that the final residue is CuO, the recorded total loss would be appropriate for the computed value based on the suggested structural formulation of the complex [Cu ($C_8H_9NO_2$)$_2$(OH)$_2$].H_2O (417.99 g/mmol) (Fig.1).

Fig. 1: structural formulation of the complex

Theoretical Calculations

We give below geometric parameters around metal in the optimized complex (Fig. 2). The analysis of the optimized geometric parameters related to the atoms involved in the coordination shows that the geometry of the complex suggests a non-regular square planar structure.

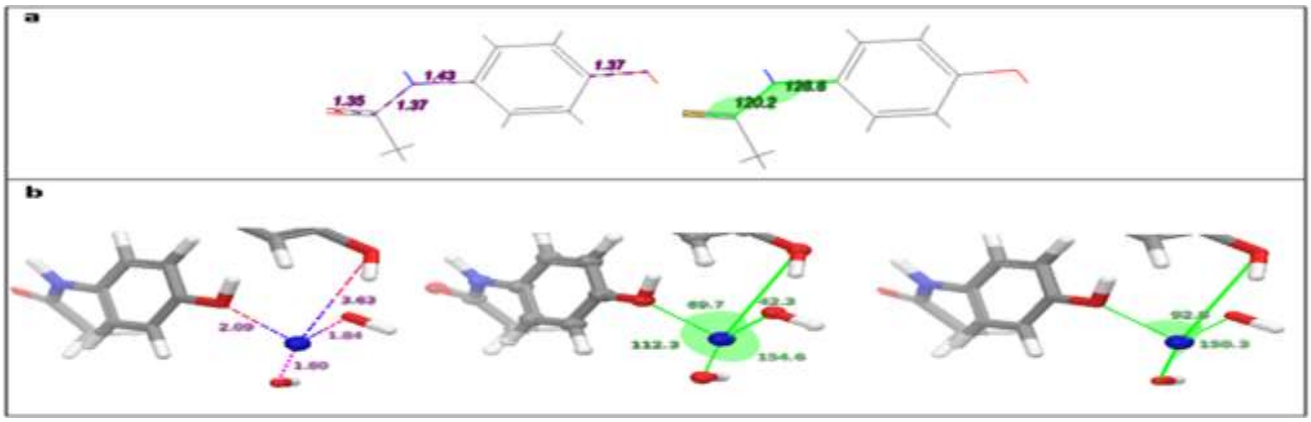


Fig. 2: Optimized geometric parameters of (a) APAP optimized at DFT/B3LYP/ and (b) Cu-APAP complex optimized by DFT/U B3LYP/ Lacvp basis. Distance (Å), bond angles are displayed in green.**

Biological Activity

Antibacterial and Antifungal Activity (in vitro)

The results of the bacterial screening of paracetamol and Cu-APAP complex are recorded in Fig. 3. No growth inhibition was observed for ethanol and copper chloride salt.

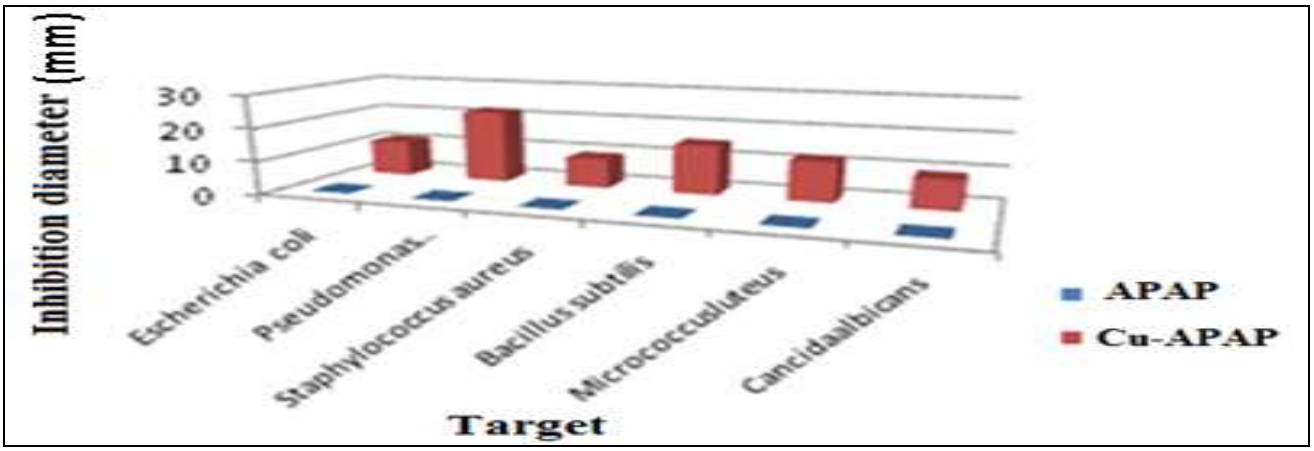


Fig. 3: Antibacterial activity of APAP and Cu-APAP complex

European harmonization [25] of antibacterial tests imposed standards to define the resistance and activity of an antibacterial agent and sets up standards for commonly used antibiotics.

Antibiotic	Inhibition zone (mm)	
Ampicilline (10 µg)	≥ 21 sensitive	< 16 resistant
Amoxicilline (25 µg)	≥ 23 sensitive	< 16 resistant

The comparison of the results obtained with Cu-APAP complex with those established for ampicilline and amoxicilline highlights the sensitivity of this compound with *Pseudomonas aeruginosa* and *Bacillus subtilis*. The results show that the metal complex is more toxic than its parent ligand against the same microorganism and under identical experimental conditions. This suggests that chelating could facilitate the crossing of a cell membrane by the complex. By the fact, the mode of action may be due to various parameters like: higher activity of the metal complex attributable to change in properties of the metal ion after complexation [26, 27].

According to Overtone's concept [28] concerning cellular permeability, the lipid membrane which surrounds the cell promotes passage of only liposoluble materials, thus lipophilicity is an important factor controlling antifungal activity. In addition, based on Tweedy's chelation theory [29], the polarity of the metal ion will be reduced because of partial positive charge sharing of the metal with donor groups present in the ligand. Thus, chelation improves penetration of complexes into lipid membranes. It is important to note that this result is in perfect agreement with the theoretical DFT study which revealed the relative high reactivity of the complex. Paracetamol and cupric complex seem inactive towards *Candida albicans* with an inhibition zone of 10 mm much lower than that obtained with Amphotericin B (29 mm), a currently antifungal medication.

Acute Toxicity

For treated groups, the present study shows that a single administration of Cu-APAP complex via the oral route up to a dose of 1140.7 mg/Kg (minimum tolerated dose (MTD)) did not produce any mortality or alter behavioral patterns in the mice as compared to the control group.

Also, no gross pathological changes were seen. The measured LD 50 value of APAP was estimated to be greater than 338 mg/Kg [30] so, the administered doses used ranged from 66.66 to 8662.18 mg/Kg.

The found LD50 is 3172.57 mg/Kg. It's obtained by the following formula:

$DL50 = DL100 - \sum (a.b) / n$. [31]

The comparison of this value with that of APAP (322 mg /Kg) shows formally that the complex is less toxic than the APAP itself with a much higher tolerance to the synthetic product.

Subacute Toxicity

Increasing doses (66.66- 8662.18 mg/Kg) of diluted Cu-APAP complex in 97° alcohol were orally administered to 10 batches of mice each consisting of 5 females and 5 males. The animals were observed for 14 days. On the other hand, it's well known that the diagram showing the variation in the weight of a laboratory animal with time is a direct means of evaluating the toxicity of the compounds administered to the animals. Examination of the different curves (Fig. 4) allows us to make the following observations:

- A constant increase in the weight of the mice in the first seven batches. This increase is similar to that of the control batch.
- For lots 8 and 9, a slight decrease in weight is recorded from the first day.
- For lot 10, 100% of the mortality occurs within twenty-four hours of the administration.

As a result, we find that batches treated with doses lesser than 50% of determined lethal dose do not affect the weight evolution of the mice. Therefore, the results obtained suggest that Cu-APAP complex is fairly non-toxic (Table 4).

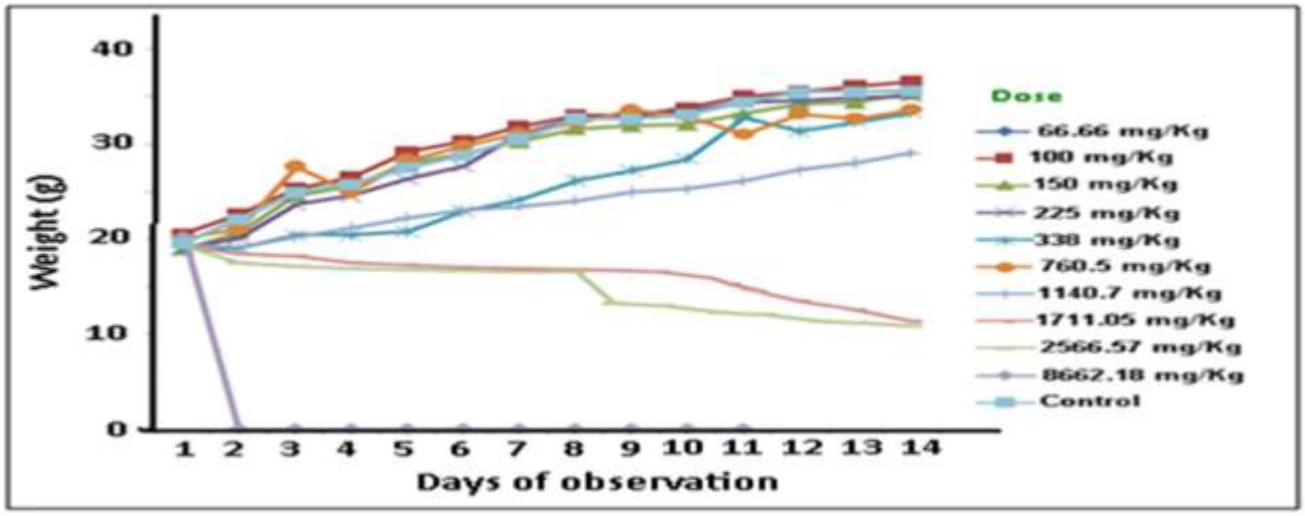


Fig.4: Variation in the weight of treated mice with Cu-APAP complex

Table 4: Results of Subacute toxicity study

Lot	Dose (mg/Kg)	Number of dead mice		Mortality (%)
		Male	Female	
1	66.66	0	0	0
2	100	0	0	0
3	150	0	0	0
4	225	0	0	0
5	338	0	0	0
6	760.5	0	0	0
7	1140.7	0	0	0
8	1711.05	3	4	70
9	2566.57	3	5	80
10	8662.18	5	5	100
Control	-	0	0	0

Anti-inflammatory Assay

The injection of Carrageenan induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates [32-34]. The study of the acute anti-inflammatory test showed that Cu-APAP complex produced a significant (P = 0.028) reduction at 4 h in Carageenan induced paw edema when compared to the positive control group (Diclofenac at 50 mg/Kg-body weight) (Fig.5). Cu-APAP exhibited 43.64 % and 66.07 % anti-inflammatory inhibition, respectively for tested doses, namely 200.1 and 400.2 mg/Kg. These values, where compared to that obtained with Diclofenac (55.73% inhibition), show clearly that Cu-APAP, when administered at relatively high doses but lower than the minimum tolerated dose (1171.05 mg/Kg) is more active than the standard. It seems that the copper-APAP binding gave an anti-inflammatory effect to the drug molecule, which thus changes the therapeutic class.

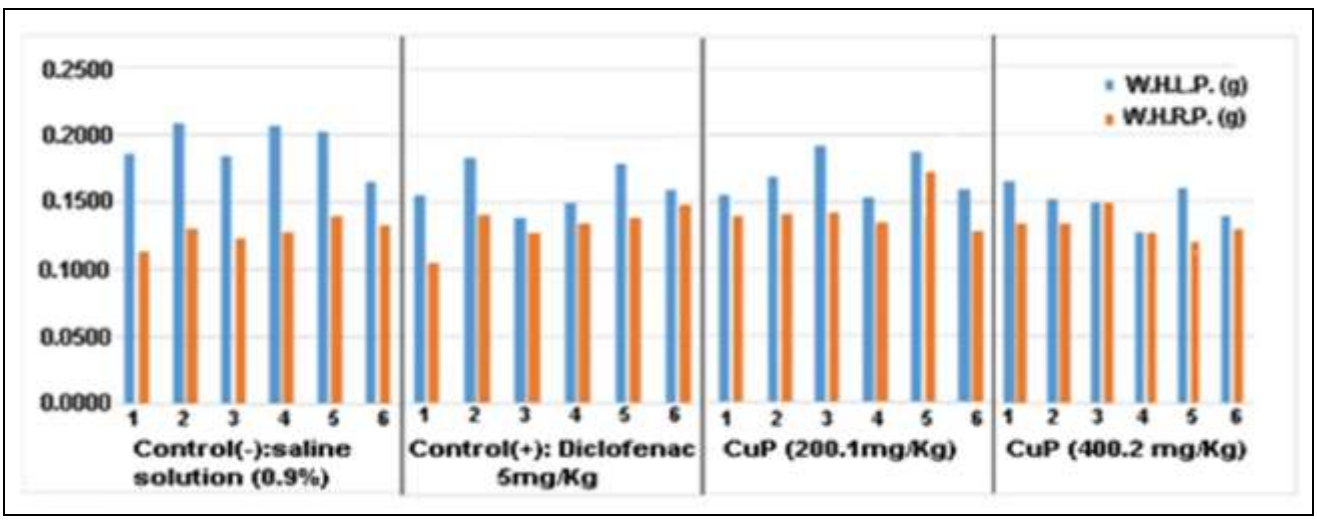
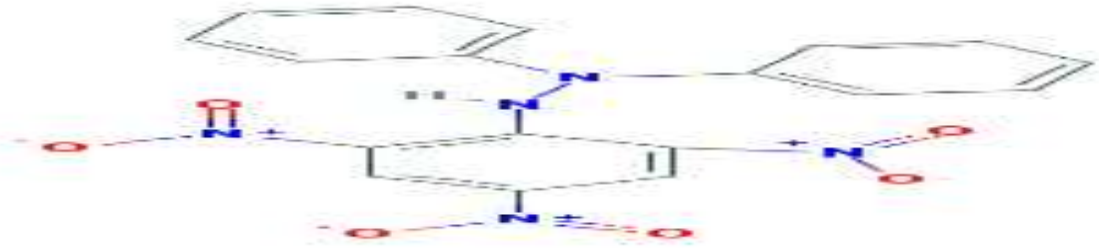


Fig. 5: Anti-inflammatory assay results

Antioxidant Assay

The antioxidant activity of APAP and its Cu(II) complex was measured in terms of their hydrogen donating or radical scavenging ability using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (DPPH: $C_{18}H_{12}N_5O_6$, $M = 394.33$) [35, 36].



Complex and free ligand antioxidant activity on the DPPH radical compared to that of a standard antioxidant (ascorbic acid) was evaluated using a UV–vis spectrophotometer by monitoring reduction of the radical which is accompanied by a change in color from violet (DPPH•) to yellow (DPPH-H).

This reduction capacity is determined through a decreased absorbance induced by anti-radical substances.

DPPH's maximum wavelength of absorption is located towards 515-518 nm in methanol. Reduction of the radical by an H donor atom leds to 2, 2'- diphenylhydrazine.

DPPH· (Violet) + AH → DPPH-H (yellow) + A·

The decreased absorbance is translates into an antioxidant activity or inhibition of free radicals in percentages (AA %) (Figure 6) using the following formula:

$$AA\% = [(A_0 - A)]/A_0] \times 100$$

A_0: bsorbance of DPPH• at t_0.
A: Absorbance of the sample.

Radical scavenging of APAP and Cu-APAP complex showed less good activities as a radical. Scavenger compared to that of ascorbic acid, which was used as a standard. However, they exhibit appreciable activity with 80% in percentage from a concentration of 300 µg/mL. A postulated mechanism for the antioxidant ability of APAP and its complex depends on the donation of the hydrogen atom of the phenol hydroxyl group which is influenced by the conjugate system resonance, the nitrogen doublet and inductive effects. The conjugation facilitates the release of hydrogen as a free radical, whereas the nitrogen pushes electron density toward the free radical, resulting in a relatively stable molecule.

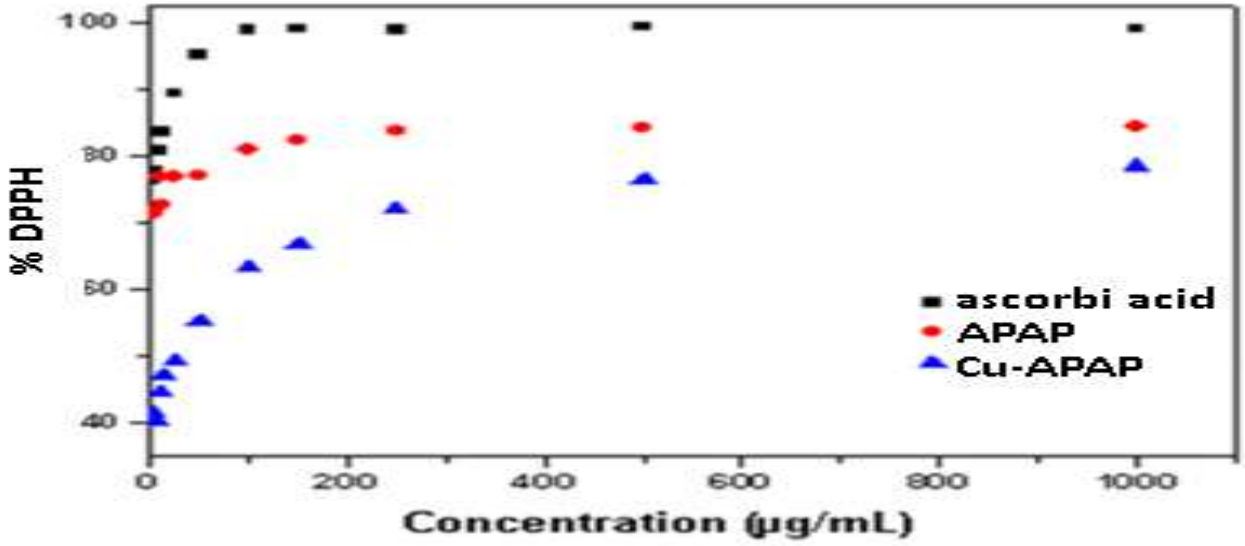


Fig. 6: DPPH scavenging activity of ascorbic acid, APAP and Cu-APAP complex

Catalase-like Activity and Kinetics of H_2O_2 Decomposition

As the subacute toxicity study shows that the complex is fairly non-toxic, we considered it advisable to evaluate its catalase-like activity at different temperatures and particularly at 37°C, the ordinary temperature of the human body. The study of the catalase-like activity (disproportionation reaction of H_2O_2 into H_2O and O_2) was carried out by volumetric determination of the evolved oxygen with a gas-measuring burette (with a precision of 0.1 mL).

All reactions were conducted in a 250 cm^3 reactor containing a stirring bar under air. Distilled water (20 cm^3) was added to the complex and the flask was closed with a rubber septum. Hydrogen peroxide (2 mL at 83.3 N) was injected through the septum with a syringe at pH 5-6. The reactor was connected to a graduated burette filled with water, and dioxygen evolution was measured volumetrically [37]. Figures 7 and 8 illustrate, respectively the effect of the temperature and the mass of the catalyst on % of evolved dioxygen during the dismutation of H_2O_2. These curves show clearly that the complex decomposes hydrogen peroxide slowly and the process reaches its paroxysm around 37° C and in the presence of 20 mg of Cu-APAP.

The decomposition of H_2O_2 was equally examined in the presence of imidazole as a nitrogen heterocyclic base adduct at 37°C. The results are illustrated in Fig. 9. The decomposition of H_2O_2 is enhanced in the presence of imidazole (imH). This will be attributed to its strong π-donating ability [38].

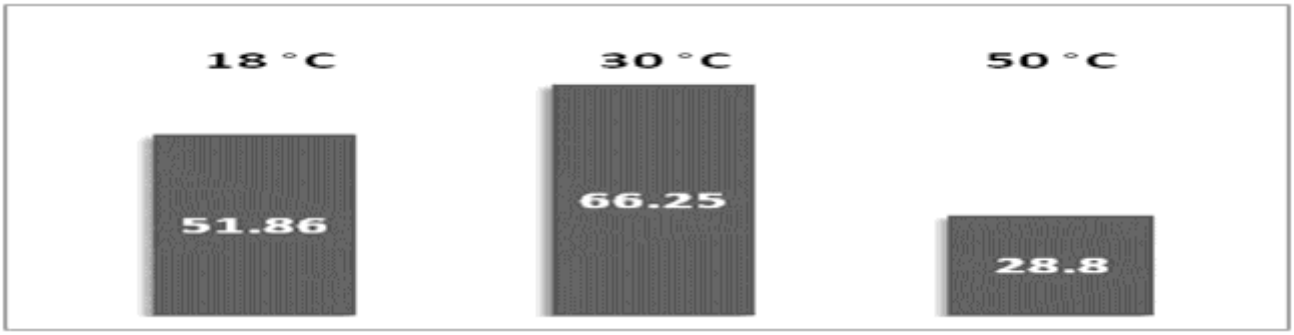


Fig. 7: Effect of the temperature on % of evolved dioxygen during the dismutation of H_2O_2 catalyzed by Cu-APAP (m = 5 mg and reaction duration = 120 mn)

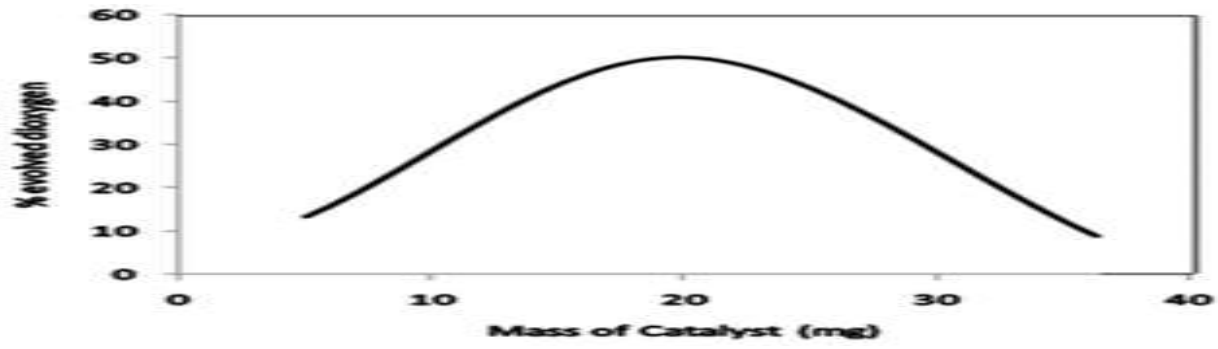


Fig. 8: Effect of the mass of Cu-APAP on % of evolved dioxygen during the dismutation of H_2O_2 (T= 37°C and reaction duration = 120 mn)

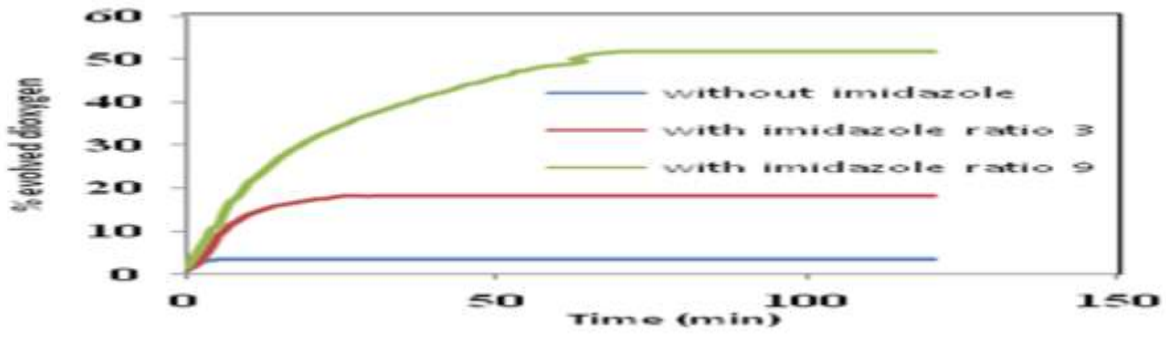


Fig. 9: Effect of addition of imidazole on % of evolved dioxygen during the dismutation of H_2O_2 catalyzed by Cu-APAP (m = 5mg, T = 37°C and reaction duration = 120 mn)

Two mechanisms could be considered. In the first one [39], the complex fixes, initially, a hydrogen peroxide molecule via an oxygen atom.

Complex + H_2O_2 → [Complex-(H_2O_2)]

While remaining fastened with the complex, homolytic rupture of the H-O- bound, releases a proton.

[Complex-(H_2O_2)] → [Complex (OOH^-)] + H^+

The second stage consists of the interaction of a second molecule of H_2O_2 with the OOH^- group carried by the metal complex to release a dioxygen molecule and a water one.

[Complex (OOH^-)] + H_2O_2 → Complex + H_2O + O_2 + OH^-

The hydroxyl ion combines with the proton released by the first molecule of H_2O_2

OH^- + H^+ → H_2O

In the second mechanism, the deprotonation of the hydrogen peroxide molecule can occurs before fixing with complex [40]

H_2O_2 → OOH^- + H^+

Complex + OOH^- → [Complex (OOH^-)]

The second stage always corresponds to the fixing of a second molecule of H_2O_2 followed by the expulsion of a water molecule, a molecule of dioxygen and an ion hydroxyl.

[Complex (OOH^-)] + H_2O_2 → Complex + H_2O + O_2 + OH^-

The hydroxyl ion combines with the proton released by the first molecule of H_2O_2

OH^- + H^+ → H_2O

Conclusion

Cu (II) complex with N-acetyl-para-aminophenol (APAP) drug has been prepared and characterized with various spectroscopic techniques and supported by DFT calculations. The IR spectral data suggested that the ligand (APAP) behaves as a monodentate coordinated ligand to the metal ions via the phenolic oxygen. Microbial studies suggested that the copper complex showed importantly raised antibacterial and antifungal activities and presented higher antimicrobial activity than the corresponding free ligand. Based on the results of the toxicological study, the acute toxicity of the complex tested on mice of NMRI strain revealed that the Cu-APAP complex is not toxic. Orally administrated complex showed a 3172.57 mg /Kg body weight LD50 value.

The anti-inflammatory assay study shows that the copper-APAP binding gave an anti-inflammatory effect to the drug molecule, which thus changes its therapeutic class. The complex acts as catalyst in the decomposition of H_2O_2. The catalytic decomposition depends upon the concentration of the catalyst, temperature and adjunction of a nitrogen heterocyclic base.

Acknowledgments

The authors would like to extend their grateful thanks to F. Benguergoura, A. Mokrani, K. T. Djebbar (Institute Pasteur de Dely Ibrahim, Alger-Algérie), A. Benabdelouahid (Département de Pharmacie, Université d'Alger-Algérie) and Dr. H. Henni (Laboratoire Nationale de Contrôle des Products Pharmaceutiques, Alger-Algérie) for technical support.

References

1. Moamen SR, Sharshar T, Khaled ME, Zein KH (2013) "Physicochemical impact studies of gamma rays on "aspirin" analgesics drug and its metal complexes in solid form: Synthesis, spectroscopic and biological assessment of Ca(II), Mg(II), Sr(II) and Ba(II) aspirinate complexes". Journal of molecular structure, 1047: 37-47.
2. Beril A (2012) Therapeutic Organometallic Compounds, Pharmacology, Dr. Luca Gallelli (Ed.), Istanbul University, Institute of Forensic Sciences, Turkiye, 652.
3. Hartinger CG, Dyson PJ (2009) Bioorganometallic chemistry- from teaching paradigms to medicinal applications. Journal chemical society reviews, 38: 391-401.
4. Kathryn LH, Katherine JF (2009) "Application of Metal Coordination Chemistry to Explore and Manipulate Cell Biology". Chemical reviews, 109: 4921-4960.
5. Lawal A, Obaleye JA, Synthesis, characterization and antibacterial activity

of aspirin and paracetamol metal complexes". biokemistri. , 2007; 19: 9-15.

6. El-Megharbel SM, Reham ZH, Moamen SR (2014) "Preparation, spectroscopic, thermal, antihepatotoxicity, hematological parameters and liver antioxidant capacity characterizations of Cd (II), Hg (II) and Pb (II) mononuclear complexes of paracetamol anti-inflammatory drug", spectrochimica acta part a: molecular and biomolecular spectroscopy, 131: 534-544.
7. Ionut L, Georgeta S, Gabriela V, Germaine S, Titus V, Lenuta M S, Calin P, et al (2013) "Synthesis and Solid-State Characterization of Zn(II)Metal Complex with Acetaminophen". revista de chimie (*Bucharest),* 64: 1127-1130.
8. Sangita PK, Farhad MA, Sania S, Zakir MS, Mohammad AH, Mohammad SA (2012) "Study of Differential Scanning Calorimetry of complex of Magnesium Sulfate with Aspirin, Paracetamol and Naproxen". bangladesh pharmaceutical journal, 15: 7-12.
9. El-Shahawy AS, Ahmed SM, Sayed NKh (2007) "INDO/SCF-CI calculations and structural spectroscopic studies of some complexes of 4-hydroxyacetanilide". spectrochimica acta part a: molecular and biomolecular spectroscopy, 66: 143-152.
10. Jaguar (2013) version 8.2, Schrödinger, LLC, New York, NY.
11. Bochevarov AD, Harder E, Hughes T F, Greenwood JR, Braden DA, Philipp DM, et al (2013) "Jaguar: A high-performance quantum chemistry software program with strengths in life and materials sciences". International journal of quantum chemistry, 113: 2110-2142.
12. Comite de(2001) l'Antibiogramme de la Société Française de Microbiologie (report 2000-2001).
13. Barry AL, Brown SD (1996) "Fluconazole disk diffusion procedure for determining susceptibility of Candida species". Journal of clinical microbiology, 34: 2154-2157.
14. National Committee for Clinical Laboratory Standards (1997) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard, NCCLS publications M7-A4, 4th Ed. Wayne Penn., 17: 2.
15. The OCDE (2006) guidelines for Testing of Chemicals, Acute Oral Toxicity- method of adjusting doses.
16. Winter CA, Risley EA, Nuss GW (1962) "Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs". Proceedings of the society for experimental biology and medicine, 111: 544-547.
17. Sharma S, Rai V, Kapoor B, Sarkar BR (2011) "Phytochemical screening and evaluation of anti-inflammatory activity of leaves extract of Holoptelea integrifolia (Roxb)". International journal of research in pharmacy and science, 1: 76-87.
18. Sagar BK, Singh RP (2011) "Genesis and development of DPPH method of antioxidant assay". Journal of food science and technology, 48: 412-422.
19. Hassaneien MM, Gabr IM, Abdel-Rhman MH, El- Asmy AA (2008) "Synthesis and structural investigation of mono- and polynuclear copper complexes of 4-ethyl-1-(pyridin-2-yl) thiosemicarbazide". spectrochimica acta part a: molecular and biomolecular spectroscopy, 71: 73-79.
20. Silverstein RM, Webster FX, Kiemle DJ (2005) "Spectrometric identification of organic compounds". 7th ed. hoboken: john Wiley & sons, .
21. Mustafa M M (2007) "Spectroscopic studies of some thiosemicarbazide compounds derived from Girard's T and P". spectrochimica acta part a: molecular and biomolecular spectroscopy, 66: 480-486.
22. K Nakamoto (1978) "Infrared and Raman spectra of inorganic and coordination compounds" Wiley online library.
23. Lever ABP (1968) "Inorganic electronic spectroscopy", Amsterdam, Elsevier.
24. Kellett A, Howe O, O'Connor M, McCann M, Creaven BS, Mc Clean S (2012) "Radical induced DNA damage by cytotoxic square-planar copper(II) complexes incorporating *o*-phthalate and 1,10-phenanthroline or 2,2′-dipyridyl". free radical biology and medicine, 53: 564-576.
25. Antibiogram Committee of the French Society of Microbiology (Recommendations 2013). Bulletin de la Société française de minéralogie. Octobre. 2004; 19: 191-193.

26. Al-Amiery AA, Al-Majedy YK, Abdulreazak H , Abood H (2011) "Synthesis, Characterization, Theoretical Crystal Structure, and Antibacterial Activities of Some Transition Metal Complexes of the Thiosemicarbazone (Z)-2-(pyrrolidin-2-ylidene) hydrazinecarbothioamide". Bioinorganic chemistry and applications, Article ID 483101, 6 pages.
27. Chohan ZH, Arif M, Akhtar MA, Supuran CT (2006) "Metal-based antibacterial and antifungal agents: synthesis, characterization, and in vitro biological evaluation of Co(II), Cu(II), Ni(II), and Zn(II) complexes with amino acid-derived compounds". Bioinorganic chemistry and applications, 2006: 1-13.
28. Parekh HM, Pansuriya PB, Patel MN (2005) "Characterization and Antifungal Study of Genuine Oxovanadium(IV) Mixed-Ligand Complexes with Schiff Bases". Polish journal of chemistry, 79: 1843-1851.
29. Tweedy BG (1964) "Plant extracts with metal ions as potential antimicrobial agents". Phytopathology, 55: 910-914.
30. Deichmann W, LeBlanc T (1943) "Determination of the approximate lethal dose with about six animals". Journal of industrial hygiene and toxicology, 25: 415-417.
31. Behrens B, Karber C "Wie (1935) sind reichenversuche fur biologische answertungen am zweckmassigsten anzuordnen Arch". Experimental pathology pharmakol, 177: 379-388.
32. Farkhanda S, Amin B, Marcel G, Gianluca C, Ulrich Florke DV, Saqib A (2010) "In vitro assessment of cytotoxicity, anti-inflammatory, antifungal properties and crystal structures of metallacyclic palladium(II) complexes"., journal of organometallic chemistry, 695: 315-322.
33. Ueno A, Naraba H, Ikeda Y, Ushikubi F, Murata T, Naramiya S et al (2000) "Lntrlnslc Prostacyclln Contrlbutes to Exudation Induced by Bradyklnln or Carrageenin: A Study on The Paw Edema Induced In Ip-Receptor-Deficient Mice". Life sciences, 66,155-160.
34. Farkhanda S, Amin B, Marcel G, Christine G, Mariyam J, de Vos D (2008) "Synthesis, characterization, *in vitro* cytotoxicity and anti-inflammatory activity of palladium(II) complexes with tertiary phosphines and heterocyclic thiolates: Crystal structure of $[PdC_{28}H_{19}N_8PS_2]$". Journal of organometallic chemistry, 693: 1117-1126.
35. Soares JR, Dinis TC, Cunha AP, Almeida LM (1997) "Antioxidant activities of some extracts of Thymus zygis". Free radical research, 26: 469-478.
36. Duh PD, Tu YY, Yen GC (1999) "Antioxidant Activity of the Aqueous Extract of Harn Jyur" (Chrysanthemum morifolium Ramat). lebensmittel-wissenschaftund- technologie, 32: 269-277.
37. Kaizer J, Csonka R, Speier G, Giorgi M, Marius R (2005) "Synthesis, structure and catalase-like activity of new dicopper(II) complexes with phenylglyoxylate and benzoate ligands", journal of molecular catalysis a: chemical, 236: 12-17.
38. Dede B, Ozmen I, Karipcin F (2009) "Synthesis, characterization, catalase functions and DNA cleavage studies of new homo and heteronuclear Schiff base copper (II) complexes". Polyhedron, 28: 3967-3974.
39. Card R J, Neckers DC (1977) "Preparation of polymer-bound bipyridine and some of its transition metal complexes". Journal of the American chemical society, 99: 7733-7734.
40. Delmond B, Jannes G (1974) "Catalysis Heterogeneous and Homogenous", Amsterdam, Elsevier, 393-406.

