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Antioxidant Investigation In Rats Administered Cobalt(Ii) Chelate Of 1-(5-Hydroxy-3-Methyl-1-Phenyl-1h-Pyrazol-4-Yl)(Phenyl)Methanone

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Abstract: *Free radical interactions with biomolecules are a process that could result in health issues like atherosclerosis, hypertension and even cancer, especially when there are insufficient antioxidants to maintain the oxidant/antioxidant balance in the body. Excessive oxidant level in the system is curbed by enzymatic and nonenzymatic antioxidants synthesized within or introduced into the system. Ascorbic acid has been, over the years, used as a standard drug for combating oxidative stress and researchers are in a continued search for a better substitute for this drug, at least one with negligible side effects. This research elucidates the antioxidant activity of 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone and its Co(II) chelate. The antioxidant property was determined in vivo by measurement of serum catalase, superoxide dismutase, and malondialdehyde levels. Serum catalase and superoxide dismutase of animals were increased by test samples while lipid peroxidation was reduced after treatment. Values were significant at ($p<0.05$). Tested compounds are therefore, recommended for further scrutiny and clinical test since they have good antioxidant potentials.*

Background: *Research into topics surrounding "oxidative stress" and redox biology has a long tradition which can be traced back to the beginning of the modern biochemistry at the turn of the twentieth century. Hitherto, researchers are in a continued quest for both synthetic and natural antioxidants, to help combat oxidative stress and consequently oxidative-stress-related diseases. We have selected this study to investigate antioxidant potentials in rats treated with 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone and its Co(II) chelates.*

Materials and Methods: *The research was done by experimental design; a total of 25 rats were used in the study. The rats were grouped into 5 groups of 5 animals in a rat cage and allowed to acclimatize for 5 days prior to the study proper. Groups I and II received the sample A (ligand) for 100 and 200 mg/kg respectively. Groups III and IV received the sample B (complex), 100 and 200 mg/kg respectively while group V took the vehicle, 5 mg/kg for the negative control. Carbon tetrachloride was used as an agent of oxidative stress at 1 ml/kg b.w at the 1st and 3rd day. Blood samples were collected from the retro-orbital plexus of the rat (ocular puncture) from where the serum was separated for baseline and after treatment analysis of the antioxidant parameters. The parameters include CAT (catalase), SOD (superoxide dismutase) and MDA (malondialdehyde) activities. After the collection of the blood samples on the 6th day, administration of the samples started on the 7th day till the 21st day (14 days) when the last blood samples were collected for final analysis.*

Results: *Results from antioxidant biomarkers (CAT, SOD and MDA) showed significant difference between tested compounds and the negative control ($p<0.05$). Samples showed good antioxidant properties.*

Conclusion: *1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone and its Co(II) complex are wonderful antioxidants. They worked very fine in vivo.*

Key Word: *Free radical, antioxidant, superoxide dismutase, catalase, malondialdehyde.*

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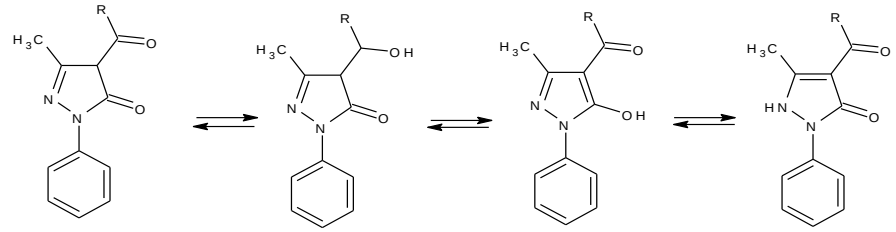
I. Introduction

Oxidation as it applies to biological systems is a biochemical reaction that can produce free radicals. Free radicals are oxygen-containing molecules with an uneven number of electrons. They are molecular species capable of independent existence that contains an unpaired electron in an atomic orbital[1].The uneven number of electrons allows them to easily react with other molecules. Although this is a normal and necessary process that takes place in the body, when there is an imbalance between free radical activity and antioxidant activity, it generally results in oxidative stress. This leads to chain reactions that may damage the cells of organisms.

Antioxidants simply try to reverse the condition of oxidative stress. They are able to achieve this because they are molecules that can donate an electron to free radicals without making themselves unstable. This causes the free radical to stabilize and become less reactive.

Ascorbic acid, the reference standard antioxidant drug for combating excessive free radical production and hence oxidative stress is been abused and causes side effects such as diarrhea, vomiting and nosea[2]. Vitamin C doses > 1 g/d elevate blood and urinary oxalic acid concentrations to a degree increasing the risk of calculi formation from calcium oxalate[3,4,5]. For this reason, researchers have carried out analysis on various substances both naturally occurring and synthetic for their antioxidant properties. Vegetables and fruits are among the natural sources of antioxidants. Polyphenols are the significant plant compounds with antioxidant activity, though, not the only ones[6]. Other plants, apart from fruits and vegetables have continuously been screened for their antioxidant properties. [7]carried out study on two plants: *pterocarpus soyauxii* and *pterocarpus santalinoides* and found the presence of fat-soluble vitamins A and E and water soluble vitamin C which are good antioxidants in both plants. [8]carried out study on the antioxidant activity of thanolic plant extracts of some *convolvulus* species using radical scavenging method. IC_{50} of 21.81 µg/ml was found for *convolvulus austroaegyptiacus* and 17.62 µg/ml was found for *convolvulus pilosellifolius.* [9]investigated and determined antioxidant properties of several medicinal plants growing wild in northern Iran. Also, a recent review had listed Several Iranian medicinal plants that have been reported for their antioxidant activities[10]. The extracts of over 20 plants in Nigeria possess marked antioxidant activity in linoleic acid model systems[11].

4-acylpyrazolone fall under the group of synthetic substances that have been undergoing scrutiny for their antioxidant property. Acylpyrazolones are interesting privileged pharmacological nucleus for bioactive and versatile ligands that are reportedly capable of coordinating to main group and transition metal ions. They form stable coordination compounds with interesting structures and posses a broad spectrum of biological activity such as antimicrobial[12], antineoplastic[13], analgesics[14] and of course, antioxidant[15]. They have been known for their easy complexation with transition metals and wide spread biological activities.



Diketo tautomer ketoenol tautomer aminodiketo

Fig 1: 3-methyl-1-phenyl-4-acylpyrazol-5-one tautomers

The present study set out to investigate the antioxidant potential of 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone and its Co(II) chelate, *in vivo* (measurement of blood serum CAT, SOD and MDA).

II. Materials and Methods

The study was done using a total of 25 male and female albino rats of wistar strain (average weight 250 g) obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Enugu State from November 2018 to February 2019.

Study Design: Experimental design and Observational study

Study Location: The study was carried out in the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Enugu State.

Study Duration: November 2018 to February 2019

Sample size: 25 rats.

Sample size calcuaton: The "resource equation method" was used to determine a suitable sample for the study. The value E for optimum sample size was gotten thus:

E = Total number of animals – Total number of groups

Subjects and selection method: The animals used in this study were obtained from The Animal House of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka, Enugu State, aged 10-12 weeks and an average weight of 250 g. They were housed in a room under controlled temperature ($26\pm2^{o}C$), Lighting (12h light/dark cycle). All rats had free access to water and standard rat chow and were handled according to standard animal ethics guidelines. The rats were divided into 5 groups of 5 animals in a rat cage and allowed to acclimatize for 5 days before the study. Suitable doses gotten from acute toxicity (LD_{50}) study of tested samples given below:

GROUP I (N= 5 rats) – Sample A 100 mg/kg b.w
GROUP II (N= 5 rats) – Sample A 200 mg/kg b.w
GROUP III (N= 5 rats) – Sample B 100 mg/kg b.w
GROUP IV (N= 5 rats) – Sample B 200 mg/kg b.w
GROUP V (N= 5 rats) – Sample C 5 mg/kg b.w (negative control)
Sample A = Ligand (1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone)(HPMBP)
Sample B = Complex (Co(II) 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone) $[Co(PMBP)_2.2H_2O]$
Sample C = Negative control (moderately saline water)

Inclusion criteria

1. Acclimatized rats
2. Either sex
3. Aged $10\leq x\geq12$ weeks
4. Oxidative biomarkers after inoculation with of CCl_4 (CAT: 31.00-36.00 μmol/mg, SOD: 185.00-204.20 U/mL, MDA: 10.00-11.60 nmol/mg)

Exclusion criteria

1. Premature animals
2. Injured animals
3. Diseased animals
4. Noticeably hyperactive animals
5. Noticeably inactive animals
6. Animals weighing less than 170 g and above 300g

Procedure methodology

After animals were ready for use, carbon tetrachloride (1 ml/kg b.w) was used to induce oxidative stress on the 1st and 3rd day. Blood samples were collected from the retro-orbital plexus (ocular puncture) on the 6th day to obtain baseline data of oxidative biomarkers. Administration of samples started on the 7th day till the 21st day(14 days), when the last blood samples were collected for final analysis. The method of[12] was used to determine the lethal dose of tested samples.

The doses of tested samples administered in the animals were as follows:

GROUP I (N= 5 rats) – Sample A 100 mg/kg b.w
GROUP II (N= 5 rats) – Sample A 200 mg/kg b.w
GROUP III (N= 5 rats) – Sample B 100 mg/kg b.w
GROUP IV (N= 5 rats) – Sample B 200 mg/kg b.w
GROUP V (N= 5 rats) – Sample C 5 mg/kg b.w (negative control)
Sample A = Ligand (1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone)(HPMBP)
Sample B = Complex (Co(II) 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl) (phenyl)methanone) $[Co(PMBP)_2.2H_2O]$
Sample C = Negative control (moderately saline water)

Catalase (CAT) activity was measured according to the[17] method. One unit of CAT activity is defined as the amount of enzyme required to decompose 1 μmol H_2O_2 in 1 min.

Measurement of SOD activity was carried out spectrophotometrically at $25^{o}C$ by the method of[18] with some modifications i.e cacodylate based buffer system was changed to potassium phosphate buffer (pH 8.0 or 9.2). One unit of the enzyme activity is defined as the amount which should produce 50 % inhibition of pyrogallol autoxidation under the standard assay conditions.

Plasma MDA level was measured by method of[19]. Values were expressed as nanomoles of MDA formed per mg plasma taking the molar absorption coefficient of MDA as 1.5×105.

Statistical analysis of data

The results were replicated three times. Mean of the values and standard deviations were calculated (mean ± SD) using Microsoft (MS) Excel program. The replicated treatments were subjected to one-way analysis of variance (ANOVA) and difference between the samples' mean were tested by Dunnett's test (multiple comparisons) using SPSS statistics software package, version 19. A $p<0.05$ was considered statistically significant.

III. Result

Table no 1 and table no 2 show the results gotten from acute toxicity test (LD_{50}). The analysis shows that the ligand doses of 10 – 1600 mg/kg b.w were safe and no mortality occurred at these doses. However, at 2900 mg/kg b.w, death was recorded. The geometric mean of the two intervals is approximately 2154 mg/kg b.w. It is expected that any dose just above the geometric mean would result in death.

The acute toxicity (LD_{50}) analysis of the complex shows that doses from 10 – 5000 mg/kg b.w were safe to work with as no death was recorded.

Table no 1: Acute toxicity study of the Ligand

Phase 1

Group	Dosage(mg/kg)	Mortality	Observation
1	10	0/3	Clustering and calm
2	100	0/3	Clustering and calm
3	1000	0/3	Clustering and calm

Phase 2

Group	Dosage(mg/kg)	Mortality	Observation
1	1600	0/1	Restless butlater calm
2	2900	1/1	Restless, dull and dead
3	5000	1/1	Restless, dull and dead

Table no 2: Acute toxicity study of the Complex

Phase 1

Group	Dosage(mg/kg)	Mortality	Observation
1	10	0/3	Clustering and calm
2	100	0/3	Clustering and calm
3	1000	0/3	Clustering and calm

Phase 2

Group	Dosage(mg/kg)	Mortality	Observation
1	1600	0/1	Mouth itching
2	2900	1/1	Mouth itching
3	5000	1/1	Restless, dull and calm

The baseline and after treatment data of oxidative biomarkers, CAT, SOD and MDA are presented in Table no 3 and table no 4. The result showed a Mean± SEM of the antioxidant/oxidative stress indexes. There is no significant difference with the negative control. The baseline was compared to the final analysis. The result shows the difference between antioxidant potential of ligand, complex and negative control which had no treatment. Values in the same row bearing the same number of asterisks are not statistically different ($p<0.05$).

Table no 3: Baseline

Samples	Dose	MDA	CAT	SOD
Ligand	100 mg/kg	11.4800±.83030*	31.0000±2.23607**	204.20±9.60417*
Ligand	200 mg/kg	10.0000±.47329*	36.0000±3.04959**	198.80±8.38093***
Complex	100 mg/kg	11.2600±.82316*	34.2000±2.97321**	191.60±5.12445**
Complex	200 mg/kg	10.5600±.74873*	35.0000±1.78885*	192.80±9.25419**
N Control	5 mg/kg	11.6000±1.24056*	36.4000±2.46171*	185.00±6.33246**

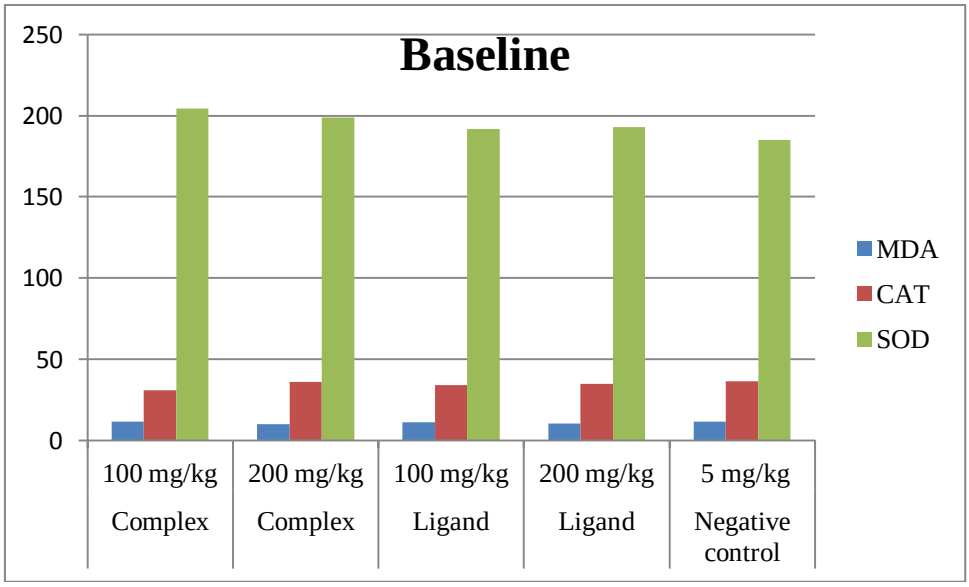
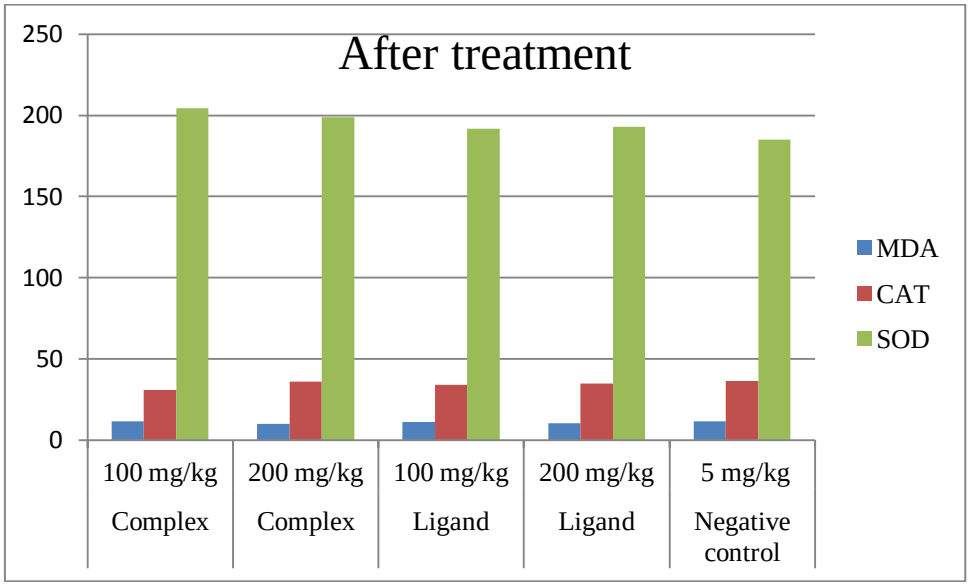


Table no 6: After Treatment

Samples	Dose	MDA	CAT	SOD
Complex	100mg/kg	10.7000±.24290*	46.8000±5.19038**	208.20±8.53464*
Complex	200mg/kg	10.3000±.32249**	43.2000±3.32265**	197.20±8.74871**
Ligand	100mg/kg	32.4000±2.41091**	45.2000±4.93356**	203.20±5.88558*
Ligand	200mg/kg	10.3600±.68235**	40.4000±4.67547**	197.60±6.99714**
N. Control	5ml/kgl	33.6400±3.41007x	67.6000±6.89638x	262.20±7.69576x



IV. Discussion

All things being equal, oxidative biomarkers balance antioxidant production in the rats. However, these parameters became biased after inoculating the rats with CCl_4. The results of CAT, SOD and MAD taken after inoculation provides the baseline for comparing the same parameters after the treatment of animals with test samples. These samples as seen in the result had good antioxidant properties; percentage changes in MDA for Ligand and Complex at 100 mg/kg each were -6.7 % and -1.8 % respectively. The changes at 200 mg/kg were 3 % and -1.89 % respectively. Percentage changes in CAT for Ligand and Complex at 100 mg/kg each were 33.7 % and 13.6 % respectively. The changes at 200 mg/kg were 16.6 % and 46.15 % respectively. Percentage changes in SOD for Ligand and Complex at 100 mg/kg each were 1.9 % and 5.7 % respectively. The changes at 200 mg/kg were -0.8 % and 2.4 % respectively.

[20]synthesized and evaluated the *in vivo* antioxidant property of novel 3-(2-naphthyl)-1-phenyl-1H-pyrazole derivatives and their results compared well with data gotten for CAT and MDA above. Cu(II) complexes with pyrazole-based ligands were seen to facilitate the conversion of superoxide anion ($O_2^{\cdot-}$) to H_2O_2 by increasing SOD activity[21]. Generally, the current results compare well with previous works done in literature and it was found that the complex was most potent in CAT and SOD, while the ligand was most potent in MDA.

To the best of my knowledge, no study has investigated the antioxidant activity of 1-(5-hydroxy-3-methyl-1-phenyl-*1H*-pyrazol-4-yl) (phenyl)methanone and its Co(II) complex in albino rats. Thus, this study aimed at widening the horizon of what is known about the pharmacological utility of acylpyrazolone derivatives.

The present study was a comparative study done in the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka from November, 2018 to February, 2019.

V. Conclusion

From the results of this study, 1-(5-hydroxy-3-methyl-1-phenyl-*1H*-pyrazol-4-yl) (phenyl)methanone and its Co(II) complex are wonderful antioxidants. They worked very fine *in vivo*. However, the molecules would have to undergo further scrutiny (clinical tests) before it can be recommended as a substitute to already existing antioxidant drugs. The current results provide good data which would serve as a platform for further studies on the 4-acylpyrazol-5-one nucleus.

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