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TOXICITY STUDY OF TWO TRANSITION METAL COMPLEXES IN SWISS ALBINO MICE

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ABSTRACT

Two schiff base complexes namely N-salicylideneglycinato-aqua-copper (II), (SGC) and N- salicylideneglycinato- di-aqua-nickel (II) dimer, $(SGN)_2$ were used to study hematological and biochemical parameters in swiss albino mice. Acute toxicity of (SGC) and $(SGN)_2$ were studied by evaluating median lethal dose LD_{50}. Hematological parameters i.e. RBC, WBC, Hb content and some biochemical parameters i.e. urea, glucose, protein, cholesterol, AST, ALT, ALP were determined by standard methods. The test compounds much below the LD_{50} values modestly altered hematological and biochemical parameters from normal values but restored gradually almost towards normal after the treatment periods. Both (SGC) and $(SGN)_2$ can be used as therapeutic agents.

Keywords: Toxicity, median lethal dose, transition metal complexes, biochemical parameters.

INTRODUCTION

Schiff bases serve as excellent chelating agents capable of forming metal complexes. Transition metal complexes containing schiff bases ligands specially the two compounds under consideration namely N-salicylideneglycinato-aqua-copper (II) and N- salicylideneglycinato- di-aqua-nickel (II) dimer $(SGN)_2$ are biologically important molecules[1]. Metal complexes of Schiff bases exhibit a wide range of pharmacological applications such as anticancer [2-5], antimicrobial [6-8], anti-inflammatory [9-10], analgesic [11] and pesticidal [12] agents.. Metal ions are generally toxic at high-dose levels. Toxicity of different mycotoxins varies greatly, depending on both their structures and the animal species; moreover some organs of the body are specially affected by the toxins. Toxic substances of blood stream are absorbed and detoxified by the liver and excreted out from the body through the kidney. Thus liver is the major site of injury for most of the toxic substances. Therefore, to study the therapeutic potential of novel metal-based compounds; the toxicity level must first be evaluated. In this context (SGC) and $(SGN)_2$ have been used to study toxic effects in swiss albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals:

All chemicals and reagents used to carry out the research work were of reagent grade.

2.2 Experimental animal:

Swiss albino mice of 5-7 weeks old, weighing 25-30 gm were collected from International Centre for Diarrhoeal Disease Reasearch, Bangladesh (ICDDR'B) Mohakhali, Dhaka (Bangladesh). Mice were kept in iron cages with saw dust and straw bedding which was changed once a week regularly. Standard mouse diet (recommended and prepared by ICDDR'B) and water were given in adequate. Protocol used in this study for the use of mice as animal model for research was approved by the University Animal Ethical Committee (27/08/RUBCMB).

2.3 Synthesis of the test compounds:

The procedure for the synthesis of the test compounds was similar to that described in the literature [13].

.2.3 Characterization:

The formation and purity of these compounds have been confirmed by taking melting points, infrared spectra, NMR and analytical data and comparing with the literature values[1,14]. Some physical properties and analytical data of the compounds have been presented in tables 1&2.

2.4 Structure of the test compounds:

The structure of the schiff base complexes are as follows:

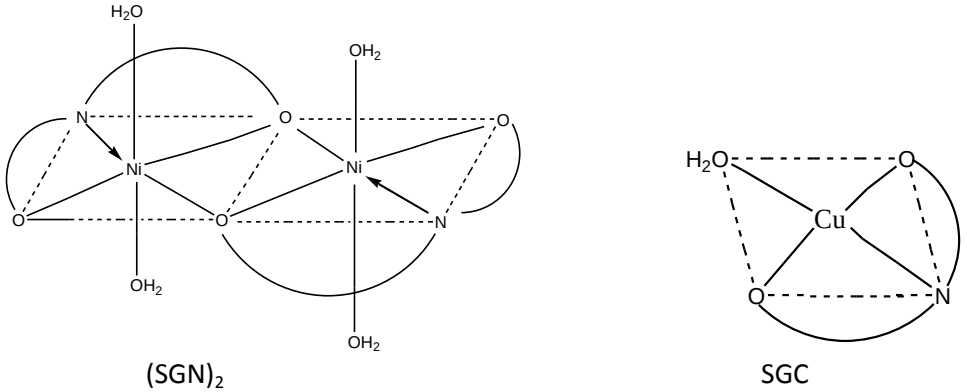
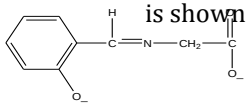


Fig.-1: Structure of the schiff base complexes.

The schiff base ligands,  is shown with the skeleton O^-–N– O^-.

2.5 Determination of median lethal doses (LD_{50}):

LD_{50} values were estimated by the "acute toxicity test" as described elsewhere [15]. The test compounds were dissolved in 3% DMSO administered orally to different groups with increasing doses. Six animals were taken in each group. Mortality was determined after 24 hours of treatment. The dose, at which the 50% mice survived, was considered as LD_{50} value of the compound.

2.6 Hematological studies:

The hematological parameters viz. white blood cell (WBC), red blood cell (RBC) and hemoglobin content were determined by standard method using cell dilution fluids and hemocytometer [16]. For this purpose blood was collected from mouse by tail puncture. Three groups (with n = 6) were taken for this purpose. Group 1 and 2 received the test compounds. Group 3 was served as untreated control. Treatment was continued for 10 consecutive days. Blood was collected on days 5, 10, 15 and 25 for the study of different hematological parameters.

2.7 Biochemical parameters

The biochemical parameters such as serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), glucose, total protein, cholesterol and urea were determined by UV-visible spectrophotometer [17-21]. Blood was collected from heart in plastic centrifuge tubes and centrifuged at 4000 rpm for 15 minutes using a WIFUNG centrifuge LABOR-50M to get clear straw color blood serum.

2.8 Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis was performed one-way ANOVA followed by Dunnett's multiple comparison test using sigma stat software (version 2.0, Jandel Scientific Inc. USA).

3. RESULTS AND DISCUSSION

LD_{50} values of (SGC) and $(SGN)_2$ were found to be 52 and 55 mg/kg *(i.p.)* of body weight respectively.

Hematological parameters were found to be altered from normal values along with treatment of the complexes. Hemoglobin content, RBC counts were found to be decreased and WBC counts were found to be increased during treatment period. Decrease in RBC and hemoglobin content result from the hemolysing power of the compounds [22-23]. The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the host [24]. After the treatment period with the test compounds, it was observed that the parameters restored almost towards normal. The effects of the test compounds on hematological parameters of are shown in table-3.

Treatment with the test compounds increased serum glucose, cholesterol, some enzymes and decreased protein and urea contents. The effect of the test compounds on biochemical parameters of swiss albino mice are shown in table- 4.

The administration of the complexes did not cause any significant changes in the biochemical parameters. The liver enzymes AST, ALT, ALP and other liver indices like total protein, glucose, cholesterol and urea contents were found to be slightly altered from normal values. As before the toxic effects as observed were found to be restored towards normal within a few days after the treatment period. This can be attributed to the temporary damage in the liver cells due to low toxic effect of the compounds [25-26].

4. CONCLUSION

Both the complexes do not cause long term toxic effect on the host. So the complexes (SGC) and $(SGN)_2$ can be used as therapeutic agents.

5. TABLES

Compound	Yield %	Physical form	Thermal stability	Solubility
SGC	60	Black crystals	Stable up to 120°C	Ethanol Methanol DMSO and Acetone
$(SGN)_2$	50	Greenish yellow crystals	Stable up to ~ 140° C	Ethanol Methanol DMSO and Acetone

Table-1: Yield percentage and physical characteristics of schiff base complexes.

Compound	Elemental analytical data found(calculated)				H_2O Content	Temp. effect from TG-DTA thermogram	IR spectra cm^{-1}
	C	H	N	Metal			
SGC	41.55 (41.93)	3.06 (3.11)	5.24 (5.44)	Cu 24.33 (24.67)	6.55 (6.98)	120°-140°C endothermic for expulsion of H_2O ~180°C exothermic for burning the organic part	1323s,1303s (C-N) 1629s,1602s (C=N) 3566sh,3550w (H_2O) 585-560w(Cu-N) 478s (Cu-O)
$(SGN)_2$	39.17 (39.75)	4.01 (4.05)	5.26 (5.10)	Ni 20.96 (21.60)	12.55 (13.25)	120°-140°C endothermic for expulsion of H_2O ~180°C exothermic for burning the organic part	3600w,3400w (H_2O) 1640s(C=N) 1307sh(C-N) 560s(Ni-N) 487s(Ni-O)

Table-2: Spectral and analytical data of the schiff base complexes.

	Day	Normal mice	Normal mice treated with schiff bases complexes (10mg/kg i.p)	
			SGC	$(SGN)_2$
RBC×10^9 cells/mL	5	5.6±0.04	3.31±0.05*	4.2±0.04***
	10		2.90±0.04	3.61±0.05
	15		4.1±0.08**	4.03±0.07*
	25		4.5.±0.08*	5.44±0.11***
WBC×10^6 cells/mL	5	10±0.82	15±0.64***	14.60±0.24**
	10		17.0±0.41*	18.03±0.22*
	15		15.15±0.15**	17.0±0.55
	25		14.0±0.41***	13.25±0.35**
Hb content mg/dL	5	11.5±0.18	8.2±0.0.06	8.81±0.22**
	10		7.0±0.10	7.43±0.35*
	15		7.5±0.02**	8.28±0.19**
	25		8.3±0.08*	9.98±0.11*

Table-3: Effect of schiff base complexes (10 mg/kg i.p.) on blood parameters of normal mice on day 9, 10, 15 and 25

Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive days. Where significant values are *p<0.05, **p<0.01 and ***p<0.001 when compared with control.

Name of Exp.	Days	AST U/L	ALT U/L	ALP U/L	Cholesterol mg/dL	Urea mg/dL	Total Protein mg/dL	Glucose mg/dL
Normal mice	0	105±0.89	58±0.36	106±0.87	150±0.42	52±0.73	7.9±0.09	145±0.57
SGC 10mg/kg	5	160±0.93***	63±0.58**	130±0.58***	165±1.08***	50.83±0.84***	7.51±0.09*	163.33±0.8***
	10	240±0.68***	79±0.57***	144±0.97***	187±0.93****	47.83±0.87*	6.78±0.02	179.5±1.08***
	15	228±1.24*	75.5±0.61***	143±0.77***	179±0.83***	47.0±0.19	6.0±0.08**	170±1.0***
	25	195±0.58***	70±0.57*	139±0.96***	175±1.01*	48.33±0.73****	7.03±0.37****	167.5±0.92*
$(SGN)_2$ 10mg/kg	5	170±0.86***	73±0.57**	123±0.96***	167±0.95****	47.83±0.87****	7.18±0.14*	200.17±0.54***
	10	236±0.73***	81±0.68*	133±0.97***	190±0.45*	46.83±0.68*	7.01±0.14**	220±0.96****
	15	230±0.86***	77.5±0.76***	128.0±0.58****	182±2.01****	46.5±0.54****	7.0±0.53****	216±1.06****
	25	205±0.57***	70±0.81**	119±0.57***	179±1.02***	47.33±0.74****	7.1±0.18***	197±0.58****

Table-4: Effect of schiff base complexes on biochemical parameters on day 5, 10, 15 and 25 at the dose 10mg/kg i.p.

Data are expressed as the mean of results in 4 mice ± S.E.M. Treatment was continued for 10 consecutive days. Where significant values are *p<0.05, **p<0.01 and ***p<0.001 when compared with control.

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