BJVAS, Vol. 1, No. 1 & 2, 42-57, 2012

Evaluation of Toxic Effect of Transition Metal Complexes on Biochemical Parameters in Swiss Albino Mice

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Abstract

In vivo acute toxic evaluation of two transition metal complexes of a Schiff base derived from salicylaldehyde and glycine, viz. $(SGCo)_2$ [N-salicylidene glycinato diaqua cobalt(II) dimer] and $(SGZ)_2$ [N-salicylidene glycinato diaqua zinc(II) dimer] were studied in Swiss albino mice. The estimated median Lethal dose (LD_{50}) of both of these complexes was 60 mg/kg (i. p.). Group 1 (n=16 mice) was treated with $(SGCo)_2$ for 10 days and Group 2 (n=16) with $(SGZ)_2$ for the same days. Group 3 (n=4 mice) was considered as control. The trial animals were monitored for 25 days providing with the same food and keeping in the same environment. Blood samples were taken from randomly selected 4 mice of each treated group on day 5, 10, 15 and 25 to study the selected biochemical parameters. The biochemical parameters were evaluated by an Automatic Analyzer (Hitachi Ltd., Tokyo, Japan using reagents of Randox Laboratories Ltd., UK). The average blood serum glucose, cholesterol, urea, triglyceride, serum alkaline phosphatase (SALP), serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) were recorded as 161.3 mg/dl, 116.4 mg/dl, 33.6 mg/dl, 117.6 mg/dl, 118.7 U/L, 59.5 U/L and 92.4 U/L, respectively in G-1; 173.4 mg/dl, 131.6 mg/dl, 37.3 mg/dl, 121.5 mg/dl, 120.6 U/L, 58.4 U/L and 95.6 U/L, respectively in G-2; 138.9 mg/dl, 103.8 mg/dl, 24.5 mg/dl, 105.5 mg/dl, 106.4 U/L, 43.7 U/L and 71.9 U/L, respectively in the control group. The observed parameters were significantly higher in the treated mice compared with the control mice at different time points of comparison ($p<0.05$). These parameters were, however identical between the two treatment groups ($p>0.05$). The estimated average total protein level was 4.6 gm/dl in G-1, 5.1 gm/dl in G-2 and 7.9 gm/dl in the control group which was lower than the normal value. The values of studied biochemical parameters were however, returned to the normalcy after 25 days of ceasing off the treatments. The obtained results indicate that, the studied metal compounds have practically no long-term toxic effects to the host.

Keywords: Biochemical parameters, Transition metal complexes, Toxicity

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1. INTRODUCTION

Toxicity of different metal complexes 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 in blood ultimately are transported to liver and excreted out from the body through kidney. Thus liver and kidney are the potential sites of injuries for several toxic substances including metal and metal complexes. Toxic substances can change the structures of endoplasmic reticulum, microsomes, mitochondria and nuclei of the hepatocytes. Therefore, these cell organelles loss the capacity of metabolic enzyme activation, protein synthesis and glucose-6-phosphate activation, leading to liver damage. Furthermore, liver damage via reactive oxygen species (ROS) pathway causes a remarkable increase of alanine amino transferase (ALT)/SGPT and aspertate amino transferase (AST)/SGOT levels in serum (Wang *et al.*, 2007). Transition metal complexes with Schiff bases have a wide range of anticancer, cytotoxicity and antimicrobial activity (Khanam *et al.*, 2006; Shrivastav *et al.*, 2003; Tumer *et al.*, 1999). For example, metal complexes with Schiff bases are being used as antitumour agent against HL-60 human leukemia cells (Tai *et al.*, 2003). $(SGCo)_2$ [N-salicylidene glycinato diaqua cobalt(II) dimer] and $(SGZ)_2$ [N-salicylidene glycinato diaqua zinc(II) dimer] complexes have already been proved to possess potent anticancer activities against Ehrlich Ascites carcinoma in Swiss albino mice (*Mus musclus*) with minimum toxicities on White blood cell (WBC), Red blood cell (RBC), haemoglobin (Hb)% etc (Ali *et al.*, 2009).

However, the effect of $(SGCo)_2$ and $(SGZ)_2$ on biochemical parameters have not been studied so far. This paper, probably for the first time in Bangladesh, describes the effects of two metal complexes - $(SGCo)_2$ and $(SGZ)_2$) on some biochemical parameters, such as SALP, SGOT, SGPT, blood serum urea etc, in Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Chemicals and reagents (cobalt-II acetate, zinc-II acetate, glycine, salicylaldehyde and di-methayl sulfoxide) used in the present study were purchased from BDH (England). Solvent (ethanol) were distilled prior to use.

2.2 Preparation of the complexes [$(SGCo)_2$ and $(SGZ)_2$]

The synthesis of the complexes was performed as per protocol described by Ray and Kauffman (1989). Briefly, alcoholic solutions of salicylaldehyde and glycine were mixed with a saturated aqueous solution of metal acetates at 1:1:1 molar ratio. The whole mixture was then refluxed for 2–3 hours in order to obtain crystalline product. The crystals were then separated from the mother liquor. The crystalline product was recrystallized from alcoholic solution for four times following the steps explained before. The final refined crystalline product was dried in an oven at 105°C for two hours and stored in a desiccator until further testing.

2.3. Characterization of the metal complexes

The synthesized metal complexes were verified by infrared (IR) spectroscopy (as potassium bromide (KBr) disc by a Shimadzu Fourier transform infrared spectroscopy (FTIR, Japan). The elemental analytical data were obtained from elemental analysis for C, H and N by using Perkin 240C analyzer and Zn and Co by an Atomic Absorption Spectrophotometer (Shimadzu, Japan).

2.4. Animals

Six to eight weeks old male Swiss albino mice, weighing 20 - 30 grams, were purchased from International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) and used to determine median lethal dose (LD_{50}) of $(SGCo)_2$ and $(SGZ)_2$ and to measure the effects of these metal complexes on biochemical parameters. The mice were fed to standard food – pellets (Ali *et al.*, 2012; Sutradhar *et al.*, 2006) and water was given ad libitum.

2.5. Determination of median lethal dose (LD_{50})

Median lethal dose represents the individual dose required to kill 50 percent of a population of animals tested (e.g., rats, fish, mice, cockroaches). Because LD_{50} values are standard measurements, it is possible to compare relative toxicities of toxic substance. The lower of LD_{50} value means the higher toxicity. The LD_{50} value was estimated as described by Plummer (1988). Shortly, the compounds of

$(SGCo)_2$ and $(SGZ)_2$ were dissolved in 2% dimethyl sulfoxide (DMSO) individually and injected to mice intraperitoneally. Each group consisted of 6 mice. The first 8 groups were treated with $(SGCo)_2$ and the rest 8 with $(SGZ)_2$. The studied doses (mg/kg) of $(SGCo)_2$ were as follows: 48 (G1), 50 (G2), 52 (G3), 54 (G4), 56 (G5), 58 (G6), 60 (G7) and 62 (G8). The studied doses (mg/kg) of $(SGZ)_2$ were 48 (G9), 50 (G10), 52 (G11), 54 (G12), 56 (G13), 58 (G14), 60 (G15) and 62 (G16). LD_{50} was evaluated by recording mortality in mice after 24 hours.

2.6. Study of Biochemical parameters

To asses serum glucose, total protein, cholesterol, triglyceride, SALP SGOT and SGPT, three groups of mice were used. Group 1 and 2 consisted of 16 mice each and these groups were treated with $(SGCo)_2$ and $(SGZ)_2$, respectively at a dose of 15 mg/kg/day (i. p.) for 10 days. On the contrary, group 3 consisted of 4 mice and this group was treated as control. One milliliter of blood was randomly collected from 4 selected mice of each group by heart puncture on day 5, 10, 15, and 25. Blood samples were centrifuged at 1500 rpm for 15 minutes. Serum samples were then stored at -80°C until further testing.

Serum glucose was estimated by Glucose-Oxidase (GOD-PAP) method (Barham and Trinder, 1972) using Automatic Analyzer (Hitachi Ltd., Tokyo, Japan) using reagents of Randox Laboratories Ltd., UK. Serum total cholesterol was measured by enzymatic endpoint method (Trinder, 1969) using Automatic Analyzer (Hitachi 704, Hitachi Ltd., Tokyo, Japan) and reagents of Randox Laboratories Ltd., UK. Urea was evaluated by the method as described by Tietz (1995). Serum triglyceride was determined by enzymatic colourimetric method. Serum alkaline phosphatase (SALP) was determined according to the method of Tietz (1994). SGOT and SGPT were studied by the method described by Bergmeyer *et al.* (1986a, b).

2.7. Statistical evaluation

Data generated from the study were entered into a spread sheet programme (Microsoft Excel Program 2003). The data were then summarized and analyzed using SPSS version 14. One factor ANOVA (analysis of variance) followed by post hoc test was applied to quantitative experimental data generated. The results were expressed as mean and standard error (SE). The level of significance was

used as 5% (0.05) cut off to interpret the results of mean biochemical parameters between the different treatment groups.

3. RESULTS AND DISCUSSION

The LD_{50} of $(SGCo)_2$ and $(SGZ)_2$ was equal of 60 mg/kg. This was relatively a high dose to cause 50% death, indicating no long-term toxic effect of them to the host (Islam, 2008). Our results of LD_{50} for the studied compounds coincide with the LD_{50} value of nickel complex with the same ligand viz. N-salicylidene glycinato diaqua cobalt-II dimer (55 mg/kg) (Ali *et al.*, 2008).

On day 10, blood serum glucose level increased by 22.4 mg/dl (16.1%) in the $(SGCo)_2$ treated mice and by 34.5 mg/dl (24.8%) in the $(SGZ)_2$ treated ones compared with the control group. However, these parameters returned to the normal values after 25 days of treatment. The detail results of serum glucose are presented in Table 1. Jesmin (2011) found that blood serum glucose level increased by 23.8 % for copper complex with the same ligand viz. N-salicylideneglycinato-aqua-copper(ll), abbreviated as (SGC) and 51.7 % for nickel complex with the same ligand viz. N-salicylidene glycinato diaqua cobalt(II) dimer, abbreviated as $(SGN)_2$ on day 10. With time progression restoration of that parameter to normalcy was also reported (Jesmin, 2011).

On day 10, serum cholesterol increased by 12.6 mg/dl (12.1%) in the $(SGCo)_2$ treated mice and by 27.8 mg/dl (26.8%) in the $(SGZ)_2$ treated ones compared with the control mice. The cholesterol level also regressed towards normalcy after 25 days of treatment. For (SGC) and $(SGN)_2$ treated groups serum cholesterol increased by 24.7 % and 26.7 %, respectively on day 10. This parameter however shows the sign of reversal towards normalcy with time (Jesmin, 2011).

Serum triglyceride increased by 12.1 mg/dl (11.5%) for $(SGCo)_2$ and by 16 mg/dl (15.2%) for $(SGZ)_2$ complex on day 10 compared with the control mice. On day 10, SALP increased by 12.3 U/L (11.6%) for $(SGCo)_2$ and by 14.2 U/L (13.3%) for $(SGZ)_2$ complex compared with the control group. Serum triglyceride and SALP were found to be restored gradually towards normalcy (Table 2). A similar result of serum triglyceride and SALP was obtained for three Zinc(II) complexes derived from 1-(2-Salicylaldiminoethyl)piperazine Schiff bases (Salga *et. al.* 2012). The SALP value was increased by 35.9 % and 25.5 %, respectively for

(SGC) and $(SGN)_2$ on day 10 which restored to the normal value within 25 days (Jesmin, 2011).

Table 1. Effect of $(SGCo)_2$ and $(SGZ)_2$ complexes on serum glucose and serum cholesterol in normal Swiss albino mice

Group	On Day	Serum glucose mg/dl blood (Mean ± SE)	Range	Serum cholesterol mg/dl blood (Mean ± SE)	Range
G0: Control group (N=4)	0	138.9±1.15	134.4-147.6	103.8±0.85	98.5-107.8
G1: Mice treated with $(SGCo)_2$ at dose 15 mg/kg (i.p.) (N=16) (4 mice were sacrificed in a day)	5	147.7±0.92***	142.6-156.3	109.2±0.66***	105.6-116.5
	10	161.3±1.06**	157.8-168.2	116.4±0.63***	109.8-121.3
	15	155.2±2.25*	146.4-167.5	111.8±1.12**	105.7-118.3
	25	141.1±1.43**	135.4-147.3	106.4±0.65***	101.5-112.4
G2: Mice treated with $(SGZ)_2$ at dose 15 mg/kg (i.p.) (G2) (N=16) (4 mice were sacrificed in a day)	5	155.3±2.50**	146.5-167.5	115.5±2.3	108.6-122.4
	10	173.4±1.80**	165.7-183.5	131.6±1.5***	127.1-138.4
	15	162.2±1.75***	154.4-170.6	126.3±2.8*	121.4-130.5
	25	145.6±1.10***	141.2-150.6	108.3±1.6***	102.6-116.8

*p<0.05, **p<0.01, and ***p<0.001compared with the control mice

The serum urea increased by 9.1 mg/dl (37.1%) for $(SGCo)_2$ and by12.8 mg/dl (52.2%) on day 10 for $(SGZ)_2$ complex compared with the control ones (Table 3). The urea level also reversed towards normal with time. The total protein value, on the other hand, decreased by 3.3 gm/dl (41.8%) for $(SGCo)_2$ and by 2.8 gm/dl (35.4%) for $(SGZ)_2$ complex on day 10 compared with the control mice. For (SGC) and $(SGN)_2$ the serum total protein value decreased by 14.18 % and 10.13 %, respectively on day 10, and was found to be restored normalcy after 25 days (Jesmin, 2011).

SGOT increased by 15.8 U/L (36.2%) for $(SGCo)_2$ and by 14.7 U/L (33.6%) for $(SGZ)_2$ complex on day 10 compared with the control mice (Table 4). SGPT increased by 20.5 U/L (28.5%) for $(SGCo)_2$ and 23.7 U/L (33.0%) for $(SGZ)_2$ complex on day 10 compared with the control mice. Salga *et al.* (2012) reported that SGOT and SGPT rose significantly in the rats for some Zinc(II) complexes derived from 1-(2-Salicylaldiminoethyl)piperazine Schiff bases. They also returned to their normal values within the next few weeks. The SGOT and SGPT increased by 128.6 % and 36.2 %, respectively for (SGC) and by 124.8 % and 39.7 %, respectively for $(SGN)_2$. They were also observed to be of normal values within the next few weeks (Jesmin, 2011).

Table 2. Effect of $(SGCo)_2$ and $(SGZ)_2$ complexes on serum triglyceride and serum alkaline phosphatase (SALP) in normal Swiss albino mice

Group	On Day	Serum triglyceride mg/dl blood		Serum alkaline phosphatase (SALP) U/L	
		(Mean ± SE)	Range	(Mean ± SE)	Range
G0: Control group (N=4)	0	105.5±1.02	101.3-112.5	106.4±0.74	103.4-108.8
G1: Mice treated with $(SGCo)_2$ at dose 15 mg/kg (i.p.) (N=16) (4 mice were sacrificed in a day)	5	110.4±0.86***	106.8-116.7	111.1±0.82***	105.4-119.3
	10	117.6±1.01***	110.8-124.6	118.7±0.98***	112.4-125.6
	15	114.4±0.61**	109.5-116.4	115.3±1.12**	106.4-122.5
	25	107.5±0.93***	98.8-116.4	109.5±1.24**	103.5-116.4
G2: Mice treated with $(SGZ)_2$ at dose 15 mg/kg (i.p.) (N=16) (4 mice were sacrificed in a day)	5	114.3±1.22**	107.2-119.6	114.8±1.08***	106.8-121.4
	10	121.5±1.52***	115.4-129.3	120.6±1.25**	116.5-128.4
	15	118.1±0.95**	113.8-125.6	117.5±1.03***	112.2-125.4
	25	109.5±1.82*	104.3-118.6	108.8±0.95**	103.4-115.8

*p<0.05, **p<0.01, and ***p<0.001 compared with the control mice

Table 3. Effect of $(SGCo)_2$ and $(SGZ)_2$ complexes on serum urea and serum protein in normal Swiss albino mice

Group	On Day	Serum urea mg/dl blood		Total protein gm/dl blood	
		(Mean ± SE)	Range	(Mean ± SE)	Range
G0: Control group (G0) (N=4)	0	24.5±0.44	22.4-27.5	7.9±0.25	7.4-8.3
G1: Mice treated with $(SGCo)_2$ at dose 15 mg/kg (i.p.) (N=16)	5	28.8±0.38***	24.8-31.5	5.9±0.62***	5.6-6.2
	10	33.6±0.54***	28.2-38.1	4.6±0.24**	4.3-4.8
(4 mice were sacrificed in a day)	15	30.5±0.92*	26.4-33.6	5.3±0.35*	4.9-5.5
	25	26.2±0.45**	23.8-29.4	6.8±0.13	6.3-7.1
G2: Mice treated with $(SGZ)_2$ at dose 15 mg/kg (i.p.) (N=16)	5	29.5±0.51**	25.8-33.1	6.2±0.62**	5.9-6.4
	10	37.3±0.22***	34.7-41.2	5.1±0.48***	4.8-5.5
(4 mice were sacrificed in a day)	15	32.4±1.12***	29.8-34.6	5.8±0.32***	5.6-6.1
	25	27.1±0.65*	24.3-29.8	7.2±0.62**	6.8-7.5

*p<0.05, **p<0.01, and ***p<0.001 compared with the control mice

Table 4. Effect of $(SGCo)_2$ and $(SGZ)_2$ complexes on serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) in normal Swiss albino mice

Group	Days	SGOT U/L		SGPT U/L	
		(Mean ± SE)	Range	(Mean ± SE)	Range
G): Control group (N=4)	0	43.7±0.84	40.6-47.4	71.9±0.52	68.7-75.4
G1: Mice treated with $(SGCo)_2$ at dose 15 mg/kg (i.p.) (N=16) (4 mice were sacrificed in a day)	5	50.2±0.64***	48.2-53.4	83.6±0.54**	78.5-88.6
	10	59.5±0.72	56.8-63.9	92.4±0.76***	87.6-98.3
	15	54.1±0.54***	51.7-57.2	86.2±1.01**	84.5-88.7
	25	46.1±0.75***	44.8-48.3	73.8±0.82*	70.2-76.8
G2: Mice treated with $(SGZ)_2$ at dose 15 mg/kg (i.p.) (N=16) (4 mice were sacrificed in a day)	5	51.1±0.48**	48.6-53.4	81.5±1.22***	78.6-85.6
	10	58.4±0.52***	57.6-62.1	95.6±0.85*	92.4-98.6
	15	53.6±0.74**	49.7-56.5	87.6±1.32***	84.1-91.5
	25	45.8±0.83***	43.1-48.4	77.2±0.92*	74.5-81.7

*p<0.05, **p<0.01, and ***p<0.001 compared with the control mice

4. CONCLUSION

The test two compounds have no significant long term toxic effects to the host. Because the two studied metallic complexes are being considered as potential agents for anticancer therapy, this study suggests that, if they are used for this purpose, the treated individuals might regain normal homeostasis within the few weeks of the withdrawal of the treatment.

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