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Anti oxidant activity of [6-chloro-2-(methylsulfanyl) pyrimidine-4-amine]cobalt(II)] complex

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ABSTRACT

Sodium nitrite is widely used as color fixative and preservative in meat and fish.Impairment of hepatic function and disturbances in lipid metabolism are well recognized advers effecte of sodium nitrite. The aim of this study is to investigate the role of [6 –chloro-2 (methylsulfanyl) pyrimidine -4-amine]cobalt(II)] complex, in preventing the oxidation of hemoglobline to methomoglobline in vitro, and preventing the disturbances in lipid metabolism induce by sodium nitrite toxicity in mice. The cobalt(II)complex of pyrimidine derivative causes inhibit sodium nitrite-induce methemoglobine formation, Moreover the complex cuases significant increase of total cholesterol, TG and LDL, while HDL significantly reduced.

Key words: Cobalt(II) complex of pyrimidine derivative, sodium nitrite, Cholesterol, Antioxidant

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INTRODUCTION

In the early 1900s, irregular curing was common place. This led to further research surrounding the use of sodium nitrite as an additive in food, standardizing the amount present in foods to minimize the amount needed while maximizing its food additive role (1), and in medicine as anti dote for cyanide poisoning (2). A principal concern about sodium nitrite is the formation of carcinogenic nitrosamines and nitric oxide via metabolites of sodium nitrite (3). About 1970, it was found that ascorbic acid (vitamin C), an antioxidant, inhibits nitrosamine formation (4). A moderate and significant acceleration of leukemia development was observed in sodium nitrite treated mice (5). Sodium Nitrite is an oxidative agent that causes methemoglobinemia in many species. Ruminants are especially vulnerable because the ruminal flora reduces nitrate to ammonia, while nitrite as an intermediate product is 10 times more toxic than nitrate. Acute intoxication is manifested primarily by methemoglobin formation and resultant anoxia (6). A major concern considering the toxicology of $NaNO_2$ is the induction of methemoglobinemia, a condition in which there is reduction in oxygen transport ability of hemoglobine (7). Various medicinal plants and vitamines protect liver from damage induce by sodium nitrite adminstrationin mice (8,9). Moreover, some pyrimidine derivatives also possess hepato- protective effect which could be attributed to their anti oxidantactivity(10). The present study was designed to investigate the roll of cobalt(II) complex of (6- chloro-2(methylsulfanyl) pyrimidine-4 amine) in preventing the reaction of $NaNO_2$ with hemoglobuline and disturbances in lipid metabolism by sodium nitrite toxicity in mice

MATERIALS AND METHODS

Nitrite-induce methemoglobuline in hemolysate:
Blood samples were collected from healthy volunteers , and centrifuged (2.500 g x 20 mins) remove plasma and the buffy coat of white cells. The erythrocytes obtained were washed thrice with phosphate- buffered saline. The washed cells were lysed by suspending in 20 vols of 20mM phosphate buffer, pH 7.4. The hemolysate was centrifuged at 25.000xg for 60 min to remove the membrane, and then diluted to give a 150 µM concentration of oxyhemoblobine. The reaction was intiated by the addition of sodium nitrite (final conc. 0.6 mM) to the solution of hemolysate and the formation of methemogloblin was measured by monitoring absorbance at 631 nm using a Shimadzu Graphicord UV 240 Spectrophotometer. Cobalt(II) complex of pyrimidine derivative was synthyesized and characterized according to the method described by Wasfi *et al* (11), cobalt (II) complex dissolved in DSMO and injected in a dose of 0.2 mg/kg body weight corresponding to 1/20 of its LD_{50}(12), and added either before or at various time –interval after the addition of nitrite. The control experiments were conducted without cobalt(II) complex and all experiments were in triplicate and were repeated many times. The data given are for one set.

Antioxidant *in vivo*
Animal and experimental design:
Mice are divided into 3 groups (5 mice in each group) as following:
1. Control group: mice were injected daily intraperitoneal with 0.2 ml distilled water, for 15 days
2. $NaNO_2$ group: mice were injected daily intraperitoneal by 0.2ml of 2.1 mg/kg $NaNO_2$ for 15 days.
3. Group three which treated by $NaNO_2$, 0.2 ml of 2.1mg/kg , i.p then after one hour the animal injected by cobalt (II) complex of pyrimidine derivative, 0.2 ml of (1/20 LD_{50}) of the complex , daily for 15 days.

At the end of the experiment period (15 days), the mice were sacrificed under light chloroform anesthesia, a 'Y' shaped cut in the mice abdomen was done , blood was collected from posterior vena cava as it enters the right ventricle(13) , blood samples was transferred into plain tubes and centrifugated at (3000 rpm for 15 minuts) to obtaun the serum which stored at -4 ^{0}C till used for measurment of different parameters.

Biochemical test:
Total cholestrol estimated by the enzymatic method described by Allian *et al* (14). Triglyceride (TG) was measured according to the method described by Fossati and prenciple (15). Associated with trainder reaction (16). HDL – cholestrol obtained in supernatant after precipitation of LDL chylomicrons from specimens by phosphotungstic acid (PTA) and mangnesium chloride was measured with total cholesterol reagent (17). Serum LDL was calculated according to Friedewald formula (18).

Statistcal analysis :
Statistical analysis was performed by a one- way ANOVA (followed by (LSD test). Data were expressed as mean ± SDM. Statistical significant was set at $p \leq 0.05$.

RESULTS

In vitro study: Nitrite causes a rapid oxidation of hemoglobin to metheoglobin . In the presence of cobalt(II) complex of pyrimidine derivative, the oxidation process was delayed in a dose dependent manner,Fig. 1. The time required to convert 50% of the available hemoglobin to methemoglobin was 6.7 min. in the absence of the complex, whereas with 20 µM cobalt (II) complex present the time was increased to 18.2 min. (Fig. 1).

Fig.1. describe the effect of cobalt (II) complex on the time –course of nitrite oxidation of hemoglobin. Without complex, the time –course of oxidation show a characteristic pattern of slow initial transformation followed by a rapid autocatalytic process. When the complex was added along with nitrite at 0 min, the formation of methemoglobin was inhibited to great extent. However , when the cobalt (II) complex was added at the end of the autocatalytic stage, i.e at 10 mins, no protection was observed. The best result in our study at concentration 2.5 µM concentration of cobalt (II) complex which protect hemoglobin from oxidation by sodium nitrite, Fig. 1.

In vivo study :

The result in Table (1) revealed that HDL- cholesterol was significantly decreased while Total cholestrol, Triglysride, LDL, significantly increased in $NaNO_2$ group compared with control group. These changes in lipid profile are reversed and their values become close to values in control group by injection of cobalt (II) complex.

Table (1): Effect of Cobalt (II) complex of pyrimidine derivative and $NaNO_2$ on lipid profile different letter denote the significant differences between groups ($P \leq 0.05$)

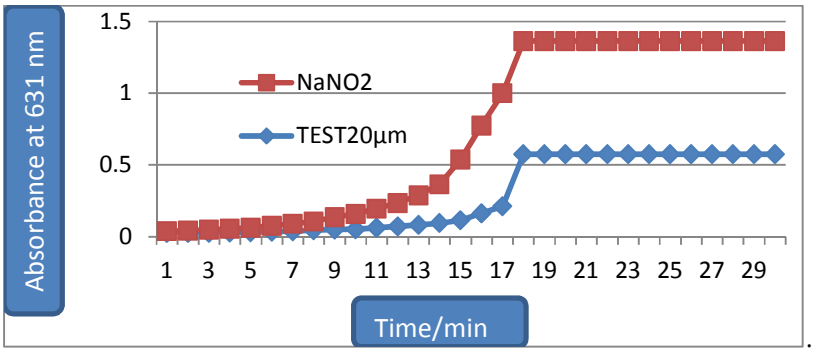
Group	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	T.Cholesterole(mg/dl)
Control	**212.60 ± 22.3b**	**53.60 ± 5.1a**	**53.88 ± 5.10b**	150.00 ± 3.80b
$NaNO_2$	**305.00 ± 25.00a**	**41.20 ± 1.30b**	**69.60 ± 7.47a**	171.80 ± 11.12 a
Cobalt (II) complex with $NaNO_2$	**203.08 ± 18.50b**	**56.80 ± 5.10a**	**56.84 ± 5.60b**	154.40 ± 4.80b
LSD	**92.40**	**17.40**	**12.40**	12.76

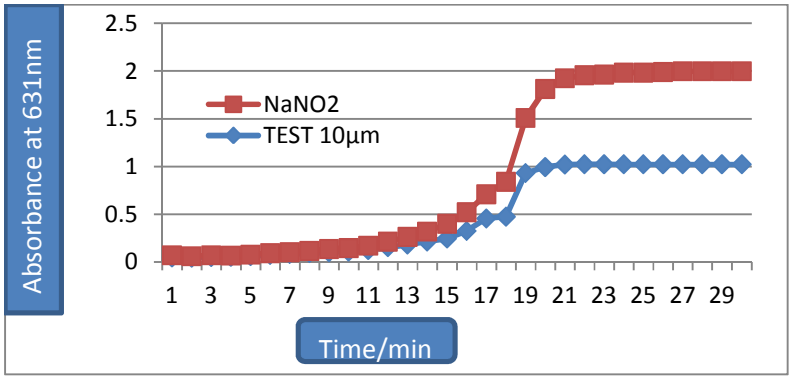
DISCUSSION

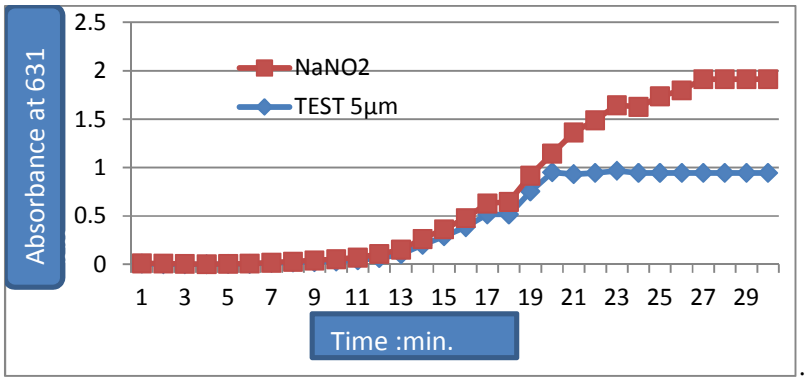
Since the cobalt (II) complex of pyrimidine derivative is newly prepared by Wasfi ,*et al* (11), with LD_{50} (192.8 mg/kg), and signifantly anti biotic activity therefore the experiments focused to determine its antioxidant activity. The present study has shown that cobalt(II) complex pyrimidine derivative can protect hemoglobin from oxidation by sodium nitrite both in hemolysate and in intact erythrocytes. Howeve, it did not to revers the effect of sodium nitrite if added at a later stage. It is well established that oxidation of hemoglobin takes place in two stages. There is slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion(19). Cobalt (II) complex is able to prevent the onset of autocatalytic stage. Since superoxide is implicated in autocatalytic stage (19) and the fact that cobalt (II) complex is a potent scavenger of superoxide (20).

Direct interaction between sodium nitrite and cobalt(II) complex pyrimidine derivative as a reason for protection is ruled out because the concentration of cobalt complex causing protection is very low (< 10µM) compared to the nitrite concentration (0.6mM). This result was disagreement with that Doyle et al (21) who used curcumin as antioxidant and recorded a 20µM. Although cobalt complex pyrimidine derivative can reduce ferric ions Fe^{3+} to the ferrous state Fe^{2+} , it fails to revers e the oxidation of hemoglobin, suggesting that protection is not due to reduction of methemoglobin to hemoglobin. Many antioxidants like ascorbic acid, uric acid, 3-ribosyl uric acid, and glutathione protect hemoglobin from oxidation by nitrite (22). These antioxidants also inhibit the onset of the autocatalytic stage of nitrite if added at a later stage(22). Thus, the effect of cobalt(II) complex pyrimidine derivative may be similar to these antioxidants in protecting hemoglobin from nitrite ions.

The best result in our study at (2.5 µM) cocentration which protect hemoglobin from oxidation by sodium nitrite (Fig.1).







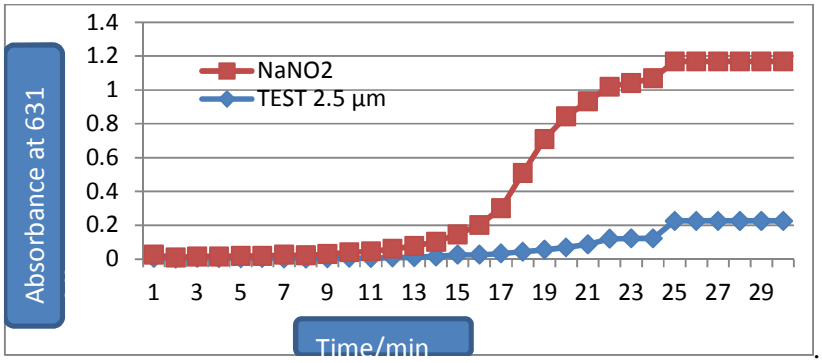


Fig.1: Antioxidant in vitro activity of Cobalt (II) complex of pyrimidine derivative at (20,10,5 and 2.5 µm)

Anti oxidant *invivo*.

Table 1. demonstrated the effect of cobalt (II) complex of pyrimidine derivative and $NaNO_2$ on lipid profile of mice after two weeks of treatment. The results demonstrated that $NaNO_2$ group showed significant increase ($P \leq 0.05$) in total cholesterol, triglyceride and LDL levels while HDL show significant decrease ($P \leq 0.05$) compared with control group, the results of treated groups shows significant decrease ($P \leq 0.05$) in total cholesterol,triglyceride , LDL and HDL levels compared with $NaNO_2$ groups. The results of this study are agreement with the results of Sidoriak and Volgin (8). The changes in lipid profile in this study may be attributed to peroxidation of cell membrane lipids and mobilization of free fatty acids from the adipose tissue to the blood stream resulted from nitrite induced oxidative stress and free radical generation. This hypolipidemic effect of cobalt (II) complex of pyrimidine derivative could be related to its antioxidant and free radical scavenging activity.

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