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Article *in* Biochemical and Cellular Archives · June 2020

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Biochem. Cell. Arch. Vol. 20, No. 1, pp. 2331-2335, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

SYNTHESIS, BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY OF OXIME DERIVATIVE ON CADMIUM CHLORIDE INDUCED MALE RATS

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(Revised 20 September 2019, Revised 29 November 2019, Accepted 29 December 2019)

ABSTRACT : The synthesis of new organic compounds has drawn the attention of organic chemists with the potential for biological and chemotherapeutic activities. New oxime derivative was prepared by 5-Bromo-1H-indole-2,3-dione with hydroxyl amine hydrochloride. In our current study, rat model was used to determine the toxicity of the synthesized compound. The up and down from Dixon has been found to have a body weight LD_{50} of 1477.2 mg/kg, moderate toxicity.

The result of physiological study was showed when the cadmium chloride was injected, there's a significant decrease in the weight of the body and organs as compared with control group, but when treated animals with new compound alone or after one hour from treated the animals with cadmium chloride there's changes in the weight of body and body weight organs, also the result was showed when cadmium injection caused significant decrease in all hormones levels as compared with control group. When treated animals with new compound alone or after 1 hour from treated rats with cadmium chloride injection, caused increase in the levels of hormones and in some hormone such as FSH there's no significant differences as compared with control .Histopathological study showed normal structure of testes, spermatogenesis and normal connective tissue in treated rats with new compound as compared to the control group. While histological changes in section of testes in rats treated with toxic substance showed loss of normal structure of testes, odema, loss both spermatogenesis and connective tissue, also the present histological study showed normal spermatogenesis and connective tissue development in the animals treated with cadmium chloride and after one hour treated with new compound.

Key words : Oxime, hydroxyl amine, acute toxicity, histopathology.

INTRODUCTION

Due to their ability to make several transformations in addition to their biological activities, oximes are essential compounds in organic chemistry.

Oximes are commonly used for aldehyde and ketone safety, purification and characterization. Oximes can also be successfully converted to amides (Bosch *et al*, 1995), nitriles (Kumar *et al*, 1999), amines (Biswanath *et al*, 1999), hydroxyamines (Das and Bhaumik, 1997), hydroxylamine O-ethers (Miyabe *et al*, 1997), nitroalkanes (Subhas Base and Vanajatha, 1998), 1,3-oxazoles, thiazoles and diazoles (Bougrin *et al*, 1998), etc. The reaction of keto esters and hydroxyl amine hydrochloride was the preparation of aromatic substituted γ- and δ-ketoxime esters (Belma, 2017). A series of compounds of pyrazoleoxime with a substituted pyridyl unit have been prepared. Displayed strong acaricidal activity against *Tetranychus cinnabarinus* in some target compounds. Several compounds have also shown strong antiproliferative activity against HepG2 cells (CuiliChen *et al,* 2017). Some steroidal oximes were prepared by Sikharulidze *et al* (2010) and its antimicrobial and anti-inflammatory activity was studied.

MATERIALS AND METHODS

Synthesis of (3*Z*)-5-bromo-3-(hydroxyimino)-1,3-dihydro-2*H*-indol-2-one

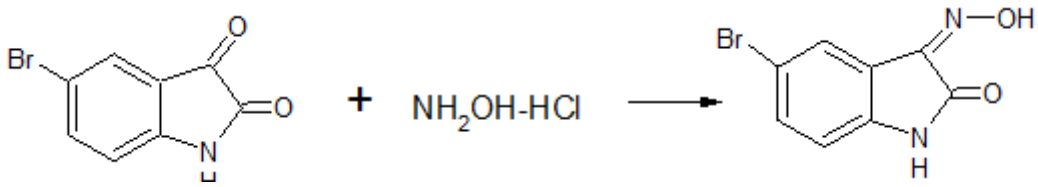
5-bromo-1*H*-indole-2,3-dione (4.4 mmol, 1.0 g) in 15 mL ethanol was added to ethanolic solution of hydroxylamine hydrochloride (4.4 mmol, 0.3 g), 10% mole of sodium acetate was added and resulting solution was refluxed for 3 h at 70-80^{0}C and then lift overnight in refrigerator. The solid product obtained was filtered and washed with ethanol and the final product was recrystallized by using chloroform: ethanol (8:2, *v*:*v*) to yield brown crystals of 5-bromo-3-(hydroxyimino)-1,3-dihydro-2*H*-indo-l2-one.

Color : Brown crystals

Yield : 82%.

M.p. : 119 -121°C.

FT-IR (KBr, ν, cm^{-1}): 3255 (OH), 3068 and 3024 (CH-Ar-H); 1710 (C=O) 1610,1635 (C=C, C=N).



Scheme 1 : Preparation of (3*Z*)-5-bromo-3-(hydroxyimino)-1,3-dihydro-2*H*-indol-2-one.

^{1}H NMR (300 MHz, $CDCl_3$, ä, ppm): 8.1 (s, 1H, C=N-OH), 7.83 (s, 1H, Ar-H_2), 7.56-7.54 (d, 1H, Ar-H_5), 6.80 (s, 1H, NH), 6.71-6.68 (d, 1H, Ar-H_6).

Acute toxicity (LD50) study

Using up-and-down method (Dixon, 1980), the lethal dose (50 percent) of the synthesized compound was calculated in rats. After performing series of test levels, male and female mice aged 4-6 weeks were injected intraperitoneally with different doses of the synthesized compound. A series of trails were performed using this approach with equal spacing between doses: increased dose after negative response and d is the tabulated value (Table 1).

Table 1 : Dixon values.

	K represented serial tests started with :				
	0	**00**	**000**	**0000**	
RXooo	0.157-	0.154-	0.154-	0.154-	OXXX
Xoox	0.878-	0.861-	0.860-	0.860-	OXXO
Xoxo	0.701	0.747	0.741	0.741	OXOX
Xoxx	0.084	0.169	0.181	0.182	OXOO
Xxoo	0.305	0.372	0.380	0.381	OOXX
Xxox	0.305-	0.169	0.144-	0.142-	OOXO
Xxxo	1.288	1.500	1.544	1.549-	OOOX
Xxxx	0.555	0.0897	0.985	1.000	OOO
	X	Xx	xxx	Xxxx	
	K represented serial tests started with:-				

Experimental animals

In the experiment 32 male rats were used, 200- 204g two months rats (*Rattus rattus*). The rats were raised in the cages in the experimental house of the college of veterinary medicine. The temperature of room was set between 21-25^{0}C and the light period. was 12 h by the use of 2 fluorescent lamps, the humidity rate was 51%.

Design of the experiment

The experiment study divided into four group (in each group 8 male), as following:

- Control group: Treated with N. S (0.9 %) in a dose of (0.5) ml via intra-peritoneal injection for 21 days.
- First treated group: Treated with oximecompound(73.86 mg/kg) about 0.5 ml via intra-peritoneal injection.
- Second treated group: Treated with Cadmium Chloride (225 mg/kg) a dose 0.5 ml via intra-peritoneal injection.
- Third treated group : First treated with (Cadmium Chloride 0.5)ml and with oxime compound (73.86 mg/kg) after one hr.

At the end of this experiment, body, testes and epididymis weight; biochemical and histopathological changes were done to evaluate the activitynew compound (oxime).

RESULTS

The present work describes the synthesis of the novel oxime derivative by 5-bromo-1H-indole-2,3-dione reaction with hydroxyl amine hydrochloride in a ratio of 1:1 to generate the oxime derivative in good yield (Scheme 1). Compound IR spectra revealed common features in some regions and distinctive fingerprint bands and other regions. The IR spectrum supports the hydroxyl group (OH) stretching with a sharp region around 3255cm^{-1}.

^{1}H NMR spectra of synthesized compounds show hydroxyl proton (OH) signals at 8.1 ppm, 1H NMR spectra of synthesized compounds show a singlet at 6.80 ppm due to (NH) and aromatic protons (Ar) signals at 6.68-7.56 ppm range.

Determination of the 50% of lethal dose (LD50) of the synthesized oxime derivative

The synthesized compound LD50 was identified in rats using the "up-and-Down" method described in (Dixon, 1980) in the experiment using 10 white mice animals 1014 weeks of age, graded oral doses for each subject, a series of concentrations (150, 200, 250, 300, 350 ... 1450) mg/k.g b.w) in 0.1 ml of dioxane was administered and selected with the same spacing (concentrations) between each animal. Before 24h, mortality was reported that each animal was treated with one dose and recorded as 0 before 24h, if the animal survives and then raised the dose being administered. While X reported for animal death and subsequently decreased the dose by the outcome of the pet, the code established as being (OOOX) and by Dixon value was obtained and the LD50 was calculated by the equation.

LD50 = Xf + Kd

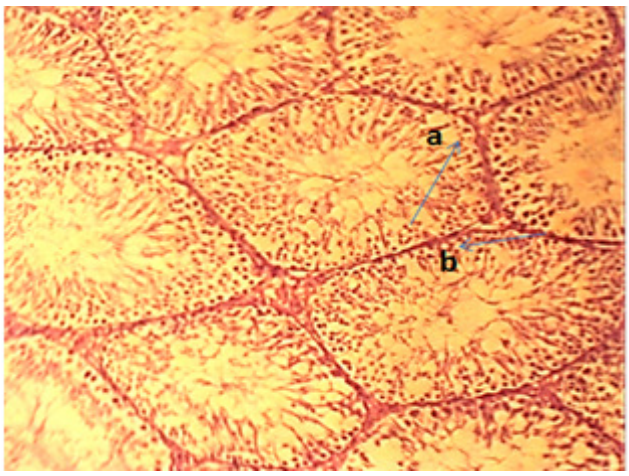


Fig. 1 : The testes of control group showed normal structure of testes, a: development of spermatogonia, b: normal connective tissue (H & E stain. 40X).

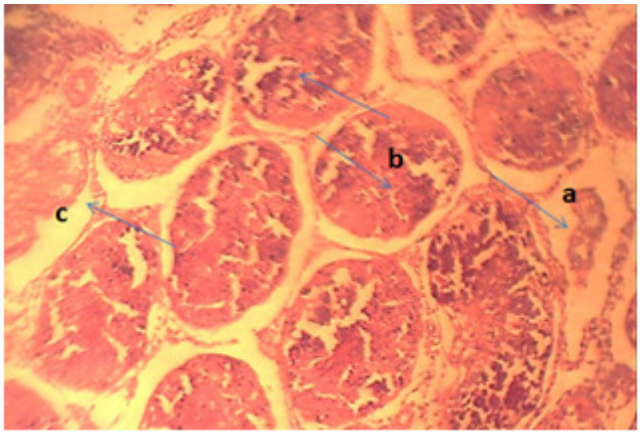


Fig. 2 : The testes of cadmium chloride group, showed loss of normal structure of testes, a : odema, b: loss of spermatogenesis development, c: loss of connective tissue.

$$LD50 = 1400 + 1.544x50$$

$$LD50 = 1477.2mg/kgb.w$$

Physiological study

Table 2 shows when the cadmium chloride was injected, caused significant decrease in body weight and weight organs (testes and epididymis) as compared with control groups, but when treated animals with new compound alone there's changes in the body weight and weight organs, also there's a significant increase in the body weight and body weight organs when treated animals with cadmium chloride and after one hour treated with new compound (oxime).

It's clear from the Table 3, cadmium chloride injection caused significant decrease in all hormones levels as compared with control group. But, when treated animals with new compound alone or after 1 hour from treated rats with cadmium chloride injection, lead to significant increase in the levels of these hormones. In some

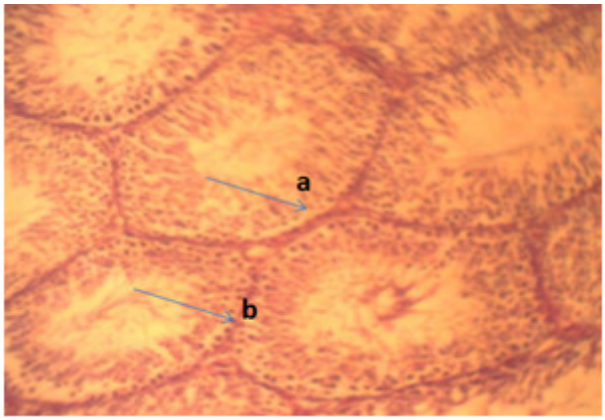


Fig. 3 : The testes of new compound, normal structure of testes, a: normal spermatogenesis, b: normal connective tissue.

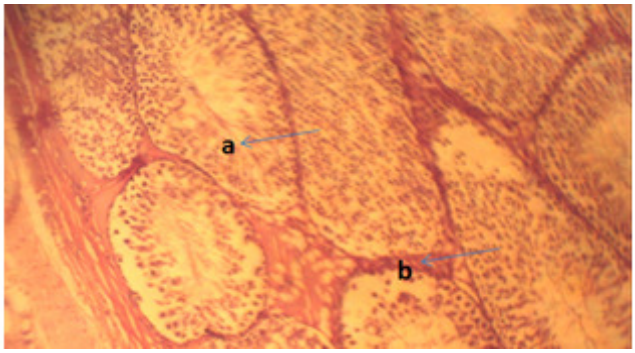


Fig. 4 : The testes of cadmium chloride and new compound. a: normal spermatogenesis development, b: normal connective tissue (H and E. 40X).

Table 2 : The effect of new compound in body and organs weight in cadmium chloride treated male rats.

Parameters\G	Body weight (gm)	Testes weight (gm)	Epididymis weight (gm)
Control group	208±6.228b	1.387±0.431b	0.718±0.008a
Cadmium chloride	189±2.483d	0.877±0.049d	0.481±0.681c
New compound	217±9.239a	1.403±0.005a	0.711±0.004a
Cadmium chlorid+ new compound	206±6.985c	1.353±0.325c	0.559±0.005b

Table 3 : The effect of new compound on hormones in Cadmium chloride, treated male rats

Parameters\G	Testosterone H(mIU/ml)	FSH, H(mIU/ml)	LH, H (mIU/ml)
Control group	4.121±0.382a	3.173±0.804a	4.128±0.354a
Cadmium chloride	2.108±0.007d	1.490±0.715c	1.436±0.488d
New compound	3.961±0.226b	3.173±065a	4.111±0.007b
Cadmium chlorid +new compound	3.153±0.016c	2.341±0.169b	3.226±0.427c

Different letters refer to significant differences among groups ($p \leq 0.05$).

hormones such as FSH there are no significant differences as compared with control group.

The results of histopathological study, showed normal structure of testes, spermatogenesis and connective tissue in treated rats with new compound as compared to the control. While histological changes in section of testes in animals treated with cadmium chloride showed loss of normal structure of testes, odema, spermatogenesis development and loss of connective tissue, also the present histological study showed; normal spermatogenesis development and connective tissue in the rats treated with cadmium chloride and after one hour treated with new compound.

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DISCUSSION

The results showed the toxic substance caused a significant decrease in the (body weight), testes, epididymis weight also cadmium chloride caused significant decrease in level of testosterone, FSH and LH hormones as compared with control group. These results were in agreement with AL-Tameemi (2010) and agreement with toxic effects of trace elements upon the hypothalamic- pituitary- testicular axis and sex hormones have been reported in recent years (Parizek and Zahor, 1956).

It's generally considered that cadmium chloride poisoning causes testicular damage (Friberg, 1957; Sokol *et al*, 1985) and histopathological changes in testicular of male rats (Pindborg, 1950). With regarded to influences on endocrine glands in the male rats, it has been reported that cadmium chloride caused reduced FSH, LH and testosterone hormones this change in this hormones in males were through to be caused by testicular damage and not to be direct effect upon the pituitary (Saksena, 1982). Toxic studies have demonstrated that may transition metals can accumulated in testes or may be caused epididymis impairing (Pandey and Singh, 2002).

Transition metals, adversely affect in spermatogenesis and cause testicular necrosis through direct effect on testicular vasculature (Ragan and Mast, 1990). Many studies reported there's decline in serum testosterone level due to inhibition of action of the steroidogenic enzymes in leydig cells (Pillai *et al*, 2011). Several transition metals including cadmium, mercury, nickel and may lead to increase (ROS) production, decrease glutathione and antioxidant levels and increase lipid peroxidation of the cell membrane, which contribute to the oxidative damage of DNA (Stohs and Bagchi, 1995; Rana, 2008). Injection of new compound to the treated rats increase (body weight) and weight of testes and epididymis and improve the level of hormones to normal level as compared to control group, the ability of new compound to improve the changes in the testes and development of spermatogenesis in treated rats and ameliorating all parameters of this study might be due to potential antioxidant and the ability of this new compound to scavenge the free radicals.

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