ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(3): 99 - 106

SJMLS - 9(3) - 010

Possible Effects of Aqueous Extracts of *Newbouldia laevis on* Haematological and Cardiac Histo-Architecture in Mercury Chloride Induced Wistar Rats.

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Abstract:
The present study investigated the possible effects of *Newbouldia laevis on* mercury induced toxicity on the haematological parameters and cardiac architecture of adult Wistar *rats. Twenty-five Wistar rats were randomly divided into five groups (A-E).* All rats had free access to feed and water. Group A served as the normal control. Group B was administered mercury chloride (10 mg/kg body weight) and a low dose (200 mg/kg body weight) of the extract. Group C was also given mercury chloride (10 mg/kg body weight) and a medium dose (400 mg/kg body weight) of the extract. Group D received mercury chloride (10 mg/kg body weight) as well as a high dose of *Newbouldia laevis* (800 mg/kg body weight), Group E received mercury chloride (10 mg/kg body weight) only**.** Extract was administered daily for 28 days. At the end of the treatment period, blood samples were obtained through cardiac puncture for haematological assay. The heart tissue was also harvested for histological evaluation. Gross morphological changes such as vascular cardiac hypertrophy and perivascular infiltration of inflammatory cells were observed in the untreated animals. Treatment with extracts of Newbouldia laevis restored the alterations recorded in some haematological parameters (red blood cell count, platelets, mean platelet volume) when compared with the untreated animals. The study has therefore shown that extract of Newbouldia laevis can be used to reduce some of the harmful effects from Mercury toxicity.

Keywords: Newbouldia laevis, Mercury, Hematology, Heart, Histo-architecture

Introduction

Contamination of the environment by heavy metals such as Mercury have affected humans and living organisms resulting in several health challenges. The increased uses of natural and artificial sources of Mercury have tremendously increased the exposure to this metal. Due to the fact that mercury like other heavy metals are not easily degradable, they accumulate in the environment and food chain (Jagadeesan et al., 2007). Report by WHO has shown that mercury in the Biosystems damages food chain and water (WHO, 1991; Clakson et al., 2006). Mercury is one of the first known toxicants, which was discovered thousands of years ago (Mandava *et al*., 2010). Different forms of mercury exist and the biological, pharmacokinetics, and clinical significance of the various forms of mercury varies (Bernhoft, 2012). Mercury is not only toxic but has high mobility especially in the ecosystem (Nascimento and Chasin, 2001). Even though there has been a reduction in mercury exposures from environmental and occupational sources, research has shown that this metal still poses a problem to the health of humans through sources like air, water and food (Vojnovic *et al*., 2004*)*. Mercury poisoning from occupational exposure actually results from long exposure and absorption of this metal over a long period of time. Mercury is used in the healthcare sector in many ways such as in vaccine preservation, in dental amalgams (consist of *50% mercury, 25% silver, tin, copper etc.), hospital equipment such as* thermometers and sphygmomanometers. *The forms of mercury include inorganic mercury (which includes* metallic mercury and mercury vapor (Hg0) and

mercurous mercury (Hg+) or mercuric mercury (Hg++) salts) and inorganic mercury. All mercury forms are considered toxic (Bernhoft, 2012). Mercury chloride ($HgCl_2$), which is the form of mercury used in this study, is highly soluble in water. After absorption mercury is found concentrated mainly in kidneys and nervous tissues. It affects various organs for example, to the intestines it causes severe acute digestive disorders, diarrhoea, intestinal mucosal injury.

There are increasing scientific data to show that environmental pollutants, such as mercury can affect the architecture of various organs in the body including the heart, as it has been reported to be easily absorbed and accumulates in nearly all organs and tissues of the body (Nielsen and Andersen, 1990). The heart is a muscular four-chambered organ that lies in the thoracic cavity whose primary function is to pump blood round the human body. The heart just like every organ in the human body can be affected by toxins which may affect its physiological, anatomical and biochemical functioning. Nowadays the exposure to this metal from both natural and artificial sources raises alarm and calls for concern. Recent studies have reported that chronic exposure to minute concentration of mercury can result in cardiovascular, reproductive, developmental, neurotoxicity, immunotoxicity and carcinogenicity. Hence the need for more scientific studies to determine the long- and short-term effects of this metal.

Newbouldia laevis is an angiosperm belonging to the family Bignoniaceae. Locally, it is called Ogirisi (Igbo), Akoko (Yoruba), Aduruku (Hausa) (Hutchison and Dalziel, 1963). Several scientific data are available that shows that the plant *Newbouldia laevis* has antioxidant and free radical scavenging, antimicrobial and antimalarial, sedative and anticonvulsant, analgesic, antinociceptive and anti-inflammatory, hepatoprotective, anticancer, uterine contraction, wound healing and antiulcer, antisickling, hypoglycemic properties (Ainooson *et al.,* 2006; Amos *et al.,* 2006; Eyong *et al.,* 2006; Joppa *et al.,* 2008; Bafor *et al.,* 2010; Hassan *et al.,* 2010; Kwete *et al.,* 2011; Owolabi *et al.,* 2011; Awemu *et al.,* .2012; Akinmoladu *et al.,* 2016).

Scientific investigations have shown that medicinal plants can be used to reduce the toxic effects of mercury in Wistar rats (Nwangwa *et al.,* 2008). The present study was designed to evaluate the effects of Newbouldia laevis on haematological, immunological parameters and to determine its effects on histological architecture of the heart in adult Wistar rats.

Materials and Methods

Plant collection and identification: The leaves of Newbouldia laevis were collected from fields around Benin City. Identification and authentication were done in the Department of Plant Biology and Biotechnology, University of Benin.

Preparation of extract: The leaves of the plant were dried for two weeks, then it was crushed into powder form. *The pulverized leaves (500grams) were extracted by soaking in distilled water (1.5L) for 48 hours with constant stirring.* Filtration was carried out using Whatman filter paper to separate the residue from the filtrate. The filtrate was then concentrated using water bath crucibles to obtain a paste like extract which was then stored in the refrigerator until needed.

Experimental animals: Twenty-five (25) adult Wistar rats (of both sexes) weighing between 110g and 200g were acquired from the Animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria, and were used for this experimental research. The rats were acclimatized for 2 weeks before commencement of the experiment and the animals were fed Top feeds grower mash and clean water. The study was approved by the Research Ethical Committee of the College of Medical Sciences, University of Benin with the approval number CMS/REC/2023/339.

The rats were randomly assigned into five (5) groups. All groups had free access to free feed. Groups A – E comprising of five rats per group.

Group A: Normal Control.

Group B: Rats induced with mercury chloride (10mg/kg body weight) + Low dosage *Newbouldia Laevis* extracts (200mg/kg body weights).

Group C: Rats induced with mercury chloride

(10mg/kg body weight) + High dosage *Newbouldia laevis extracts* (400mg/kg body weight).

Group D: Rats induced with mercury chloride (10mg/kg body weight) + High dosage *Newbouldia laevis extracts* (800mg/kg body weight).

Group E: Rats induced with mercury chloride (10mg/kg body weight) only.

Sample collection: At the end of the treatments period (28 days), rats were weighed and then sacrificed under chloroform anaethesia. Blood samples were collected into EDTA anticoagulant sample bottles for haematological analysis and the heart of each rat was harvested and immediately fixed on 10% formalin to avoid autolysis and used for histopathological evaluation. The haematological parameters were analysed using the hematological auto analyser.

Photomicrography: The section of the heart were obtained and examined under Leica DM750 research microscope with a digital camera attached. Digital photomicrgraphs of the tissue sections were taken at ×400 objective magnifications. Cardiosomatic index was calculated using the equation below

Cardiosomatic index = (Heart weight / Body weight) x 100.

Statistical Analysis: Data were subjected to statistical analysis using the IBM SPSS statistical software version 25. One-way analysis of variance (ANOVA) was carried out and data were represented as mean ± SEM. Values of $p \leq 0.05$ were considered significant.

Results

Effects of Newbouldia laevis on body weight of mercury induced Wistar rats

Table 1 depicts the effect of *Newbouldia laevis* on body weight of mercury induced Wistar rats. The result shows a significant increase in the final weight of the animals when compared with the initial weight. However, A higher percentage increase in body weight was recorded for the high dose treated rats (Group D) when compared with other groups.

Table 1: **Effects of *Newbouldia laevis* on body weight of mercury induced Wistar rats**

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group A	1 1 0.60±2.29	1 74.00±7.80*
Group B	1 26.80±7.1 2	1 69.80±1 2.48*
Group C	1 32.00±9.73	1 53.60±5.55*
Group D	1 43.80±8.35	200.40±9.98*
Group E	1 34.67±1 1 .57	1 82.00±7.81 *

*Values are mean ±SEM (n=5) *significantly different from the control group*

Effects of *Newbouldia laevis* on cardiac weight and cardiosomatic index of mercury-induced Wistar rats

Table 2 depicts the effects of *Newbouldia laevis* on cardiac weight and cardiosomatic index of mercury induced Wistar rats. The result showed that with the exception of group D, a nonsignificant increase was recorded for the cardiac weight and cardiosomatic index for all groups when compared with the control ($p \leq 0.05$).

Table 2: Effects of *Newbouldia laevis* on cardiac weight and cardiosomatic index of mercury induced Wistar rats

Groups	Cardiac weight(g)	Cardiosomatic index (%)
Group A	0.52±0.02	0.30±0.01
Group B	0.58±0.09	0.33±0.02
Group C	0.52±0.04	0.34±0.02
Group D	0.76±0.08*	0.38±0.03*
Group E	0.60±0.06	0.33±0.03

Values are mean ±SEM (n=5) *significantly different from the control group

Effects of *Newbouldia laevis* on hematological indices in mercury induced Wistar rats

Table 3 depicts the effects of *Newbouldia laevis* on hematological indices in mercury induced Wistar rats. Non-significant change in white blood cell count, lymphocyte, granulocyte, hematocrit, and hemoglobin count for the treated and untreated animals when compared with the normal control rats. While significant alterations were observed for the red blood cell count, mean corpsular volume, mean corpuscular hemoglobin, platelet and mean platelet volume for the untreated animals when compared with the normal control. Treatment ameliorated the alterations in some of these markers.

Table 3: **Effects of *Newbouldia laevis* on haematological indices in mercury induced Wistar rats**

	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
WBC (1 0^3/µL)	6.98±1 .37	8.95±1 .1 8	8.38±0.82	7.44±0.84	8.20±0.75
Lymphocytes (%)	90.00±1 .80	89.05±2.26	91 .35±1 .30	84.1 8±0.81 *	90.90±1 .1 2
MID (%)	7.1 3±1 .1 8	1 1 .98±1.53*	6.1 3±0.93	1 1 .42±0.63*	6.73±0.82
Granulocytes (%)	2.88±0.68	3.98±0.78	2.53±0.40	4.40±0.30*	2.37±0.30
RBC (1 0^6/µL)	7.48±0.1 1	7.21 ±0.1 5	7.1 8±0.21	7.41 ±0.1 2**	6.52±0.29*
Haemoglobin (g/dL)	1 4.68±0.20	1 3.88±0.1 5	1 3.65±0.33*	1 4.08±0.30	1 4.30±0.1 2
Haematocrit (%)	45.28±0.74	44.63±0.97	47.68±2.1 3	44.76±1 .1 2	46.30±1 .1 3
Mean Corpuscular Volume(fL)	60.68±1 .24	62.1 5±2.29**	66.53±2.23**	60.52±1.09**	71 .43±3.81 *
Mean Corpuscular Haemoglobin (pg)	1 9.60±0.49	1 9.20±0.20**	1 9.00±0.63**	18.98±0.22**	22.00±1 .21 *
Mean Corpuscular Haemoglobin Concentration (g/dL)	32.35±0.53	31 .1 0±0.93	28.68±0.83*	31 .42±0.32	30.87±0.66
Platelets (1 0^3/µL)	534.25±83.11	576.75±50.71 **	401.00±75.25 **	491.60±64.68 **	579.00±111.50*
Mean Platelet Volume (fL)	7.1 8±0.15	7.30±0.11	7.43±0.09*	7.48±0.32*	7.93±0.29*

Values are mean ±SEM (n=5) *significantly different from the control group, **significantly different from the untreated group (group E)

Effects of *Newbouldia laevis* on histology of the heart tissues in mercury treated rats

Fig 1 shows the photomicrographs of the heart tissue of the control and the *Newbouldia laevis* treated rats. Gross morphological changes in the heart were observed for the untreated animals while treatment with *Newbouldia laevis* ameliorated these observed changes.

Fig 1: Photomicrographs (plates 1-5) of heart tissue of *Newbouldia laevis treated mercury induced rats.*

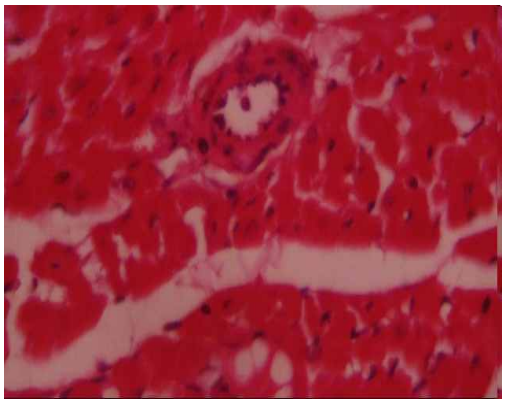

Plate 1. Rat heart. Control. Composed of normal tissue: A. bundles of cardiomyocytes. (H&E x 400)

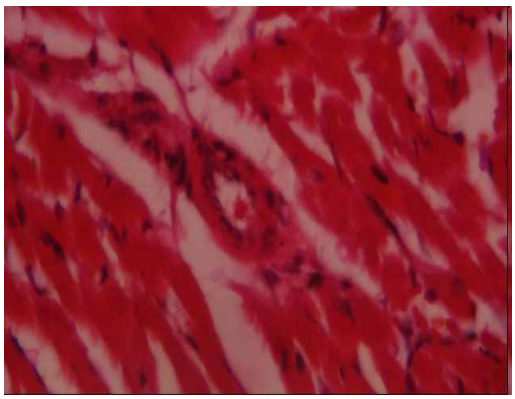

Plate 2. Rat heart given HgCl2 + low dose extract showing normal architecture: A. bundles of cardiomyocyte, B. coronary artery, C. interstitial space (H&E x 400)

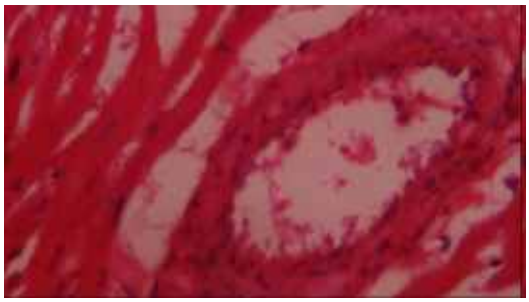

Plate 3: Rat heart given $HgCl_2$+ medium dose extract showing normal architecture A. bundles of cardiomyocytes, B: interstitial space, C, coronary artery (H&E ×400).

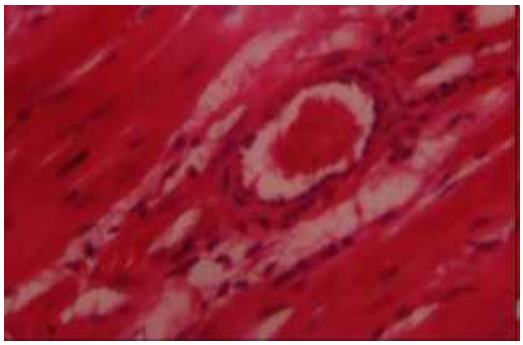

Plate 4. Rat heart given $HgCl_2$ + high dose extract showing: A. normal bundles of ca rdiomyocytes, B. coronary artery, C. perivascular infiltrates of inflammatory cells (H&E x 400).

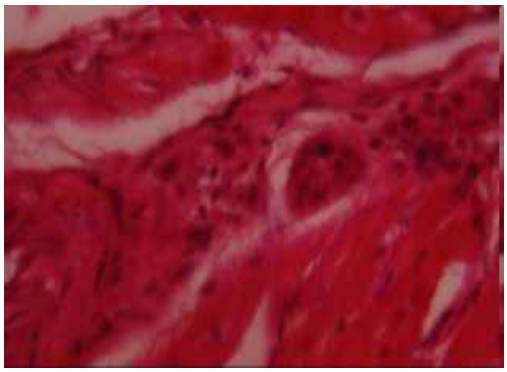

Plate 5. Rat heart given HgCl2 only showing: A. vascular hypertrophy B perivascular infiltrates of inflammatory cells (H&E ×40)

Discussion

Research investigating the effects of hazardous heavy metals on biochemical and physiological markers are increasing. This is primarily due to the increased usage of these toxicants in the industrial, domestic, agricultural and medicinal sectors of the economy. Owing to the known detrimental health effects on living organisms and biological systems in the ecosystem, it is therefore of vital importance to monitor the effects of these toxicants on the biological system.

The impact of a toxicant on a biological system can be monitored by evaluating the haematological and biochemical biomarkers of the living organism (Li *et al.,* 2010). Haematological parameters such as white blood cell count, hemoglobin, platelet, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin concentration are useful parameters in evaluating the toxic stress, integrity of the immune system and tissue damage (Nemcsok and Benedeczky 1990; Talas and Gulhan 2009; Kavitha *et al.,* 2010). These markers are easily affected by environmental or physiological factors, so quantifying their levels will provide a measure of the physiological changes in the organisms (Remyla *et al.,* 2008).

No adverse effect of mercury chloride on the body weight and organ weight was recorded in this study. Our findings also agree with the report of Ajibade *et al.* (2019) who reported than administration of mercury chloride to adult Wistar rats did not adversely affect their body weight.

In this study, we recorded a significant decrease in red blood cell count for the untreated groups (group treated with mercury chloride only) when compared with control group. Only treatment with high dose *Newbouldia laevis* significantly increased the red blood cell count. Several studies have established that treatment with mercury chloride results in reduction of red blood cell count (Al-Zubaidi and Rabee, 2017). Our present result agrees with Brandao *et al.,* (2018) who established that mercury exposure caused a reduction in the erythrocyte count.

The white cell count, red cell count, and platelet count are quantitative evaluations of blood constituents. Haemoglobin (Hb) and hematocrit (HCT) are indicators of the oxygen- conveying potential of blood. The non-significant changes recorded for the haematocrit and haemoglobin concentration in this study is not line with some studies on toxicological effects of mercury (Shah and Altindag 2004; Aliakbar and Zahra, 2013).

Using lymphocyte and granulocyte counts as a marker for immune status, treatment with mercury chloride did not alter the immune status of the Wistar rats. Only the groups treated with high doses of *Newbouldia laevis* showed a significant decrease in the lymphocyte count when compared with the untreated groups.

Platelets are the smallest blood cells and sensitive marker for health disturbances (Peng *et al.,* 2004). Estimation of platelet count provides details on platelet activation, and this is indicative of the presence of diseases prone to inflammation. We recorded a significant increase in platelet counts, mean platelet volume for the untreated animals. This result is in line with that observed by Hounkpatin *et al.* (2013) who reported on the haematological evaluation of Wistar rats exposed to doses of cadmium and mercury. Treatment with varying doses of extracts of *Newbouldia laevis* resulted in a significant decrease in the platelet count.

The major findings during Histological examination of the heart are usually well-defined cardiomyocytes and connective tissue. Cardiomyocytes are the cells of the heart that function mainly in the contraction of the heart, by utililizing a fine network of contractile proteins for this role. For the untreated groups, significant vascular hypertrophy was observed, and this could have a tremendous impact on the gross structure and physiology of the heart and its function. Treatment with low, intermediate and high doses of *Newbouldia laevis* treated rats revealed a well- defined cardiomyocytes, interstitial spaces and coronary artery in the photomicrographs of these groups.

Conclusion

This study has therefore shown that mercury chloride can cause significant alterations in some haematological parameters and gross changes in

the architecture of the heart. Treatment with extracts of *Newbouldia laevis* can be used to reduce some of the toxic effects of mercury chloride in adult Wistar rats.

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Citation: Innih, S.O., Nwachukwu, J.U. Possible Effects of Aqueous Extracts of *Newbouldia laevis* on Haematological and Cardiac Histo-Architecture in Mercury Chloride Induced Wistar Rats. ***Sokoto Journal of Medical Laboratory Science*; 9(3): 99 – 106**. https://dx.doi.org/10.4314/sokjmls.v9i3.10