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Discovery

Impairment of cardiovascular function indices in male rats induced by aluminium-tainted water: Atherogenic indices and predictor ratio assessment

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Cardiovascular disease (CVD) is globally increasingly becoming public health concern. There is increasingly growing evidence that exposure to metal pollutants are risk factors that disturbs lipid metabolism with increase risk for CVD. The present work aimed to undertake to evaluate the toxicity of aluminium chloride - tainted drinking water ($AlCl_3$) on lipid profile in male wistar rats in assessing cardiovascular risk by using atherogenic indices and prediction ratio. Fifty male wistar rats were randomly assigned to five groups of 10 rats each. Control group was given normal drinking water whilst $AlCl_3$ treated groups received 200-800mg/kg $AlCl_3$ orally once daily for 28 days. Thereafter, blood samples were collected for lipid profile analysis. Atherogenic indices like Castelli's Risk Index (CRI), Atherogenic Index of Plasma (AIP), and Atherogenic Coefficient (AC), lipid ratios and predictor ratios were calculated. Overall, the estimated atherogenic indices- CR1-11, AC and CRI-1, the ratios TG/HDL-c, TC-HDL-c/HDL-c, TC/HDL-c and low HDL-c/LDL-c, rose strongly dose-dependently approximately and significantly different determine cardiovascular risk in rats by $AlCl_3$. Predictor ratio however, revealed that AIP did not significantly impact the risk of cardiovascular disease in rats. In conclusion, exposure to $AlCl_3$ elicited concurrently dose-response proliferation of both dyslipidaemia and atherogenic indices differentially with resultant deleterious effect in cardiovascular cells and tissues in rats. AIP may not be an independent factor in $AlCl_3$ impacting the risk of CVD in rats. These might be the various possible mechanisms of aluminium toxicity in male rat cardiovascular risk.

INTRODUCTION

Cardiovascular disease (CVD) epidemic is an ever-growing health problem and remains the leading cause of death in all regions of the worldwide. Exposure to heavy metals is unavoidable, and in general, is associated with disturbed lipid metabolism with an increase in the prevalence of dyslipidaemia for cardiovascular risk [1-5]. Heavy metals are commonly defined as those having a specific density of more than 5 g/cm^3. One of such is aluminium. Aluminium is the third most prevalent element and the most abundant metal in the earth's crust, only oxygen (49.5%) and silicon (26%) occur more commonly than aluminium (8%) [6]. It serves no biochemical role in the body but is highly biologically reactive and its accumulation in tissues and organs are associated with serious health problems [7-13]

The potential association between aluminium and increased cardiovascular risk factors are less well defined and are still unclear, however, impaired antioxidants metabolism and oxidative stress has been implicated to play some crucial role [1, 13]. Recently, atherogenic indices - Castelli's risk indexes (CRI-I) =TC/HDL-c, (CRI-II) =LDL-c/HDL-c, Atherogenic Coefficient (AC) = (TC–HDL-c)/ HDL-c or TG/HDL-c ratio, and Atherogenic Index of Plasma (AIP) =log (TG/HDL-c) has been suggested as powerful indicator of the risk assessment of cardiovascular diseases beyond the conventional lipid parameters. Interestingly, it has been argued that the indices could be useful predictor at higher risk of cardiovascular disease especially, when the absolute values of lipid profile seem normal or higher and not markedly deranged or in centres with insufficient resources [14-20] , Furthermore, predictor ratio model [13] that explores the interplay of interrelationship between damaged tissue biomarkers has also been suggested as a sensitive and specific predictor of the development of physiopathological risks.

The paucity of data regarding the relationship between atherogenic indices, lipid ratios and predictor ratios and aluminium toxicity and the risk of cardiovascular disease (CVD) warranted us to conduct this study, aimed to evaluate the importance of these indices in male wistar rats in determines cardio vascular risk, which has not so far been studied.

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MATERIALS AND METHODS

Approval of institutional ethics committee was obtained. A total of fifty healthy local breed male [3] wistar rats weighing 100-120g were randomly assigned to five groups of experimental groups (10 rats in each) as follows: Group 1 was given 2ml of water/day. The doses of $AlCl_3$ were calculated according to animal's body weight before treatment. Groups 2-5 were given 200, 400,600 and 800mg/kg/body weight per day of aluminium chloride-tainted drinking water ($AlCl_3$) orally, respectively. The animals were housed in separate cages and fed with standard rat chow and water *ad libitum*. All rats were handled in accordance with standard guide for the care and use of laboratory animals.

Collection of blood samples, estimation of lipid profile and cardiac risk indices

The treatment period lasted for a total duration of 28 days to ensure delivery of aluminium to target sites. Thereafter, the animals were humanely scarified using diethyl ether anaesthetic agent and followed by cervical decapitation. Blood samples were taken for measurement of lipid profiles - low-density lipoproteins (LDL-c), high-density lipoproteins (HDL-c), triglycerides (TG) and total cholesterol (TC). The tests were carried out in an automated clinical auto analyzer. After the estimation of TC, TG and HDL-c, the risk indices LDL-c, were calculated using Friedewald formula [21] as follows: LDL-c =Total cholesterol – [(TG/5) +HDL-c] and atherogenic index (AI) = (TC-HDL-c /HDL-c).

Cardiovascular risk indices - atherogenic indices like Castelli's Risk Index-I (CRI-I)=TC/HDL-c; Castelli's Risk Index-II (CRI-II) = LDL-c/HDL-c; Atherogenic Coefficient (AC) = {(TC – HDL-c)/HDL-c}; and Atherogenic Index of Plasma (AIP) = $\log_{10}$ (TG/HDL-c) ratio were derived via manipulation of the various lipid profile results obtained using defined mathematical calculations [14-20].

Statistical Analysis

SPSS version 20.0 was utilized to analyze the data. One Way Analysis of Variance (ANOVA) was used as the statistical tool and the results were presented as mean ± SEM. Student's T-test was applied for further comparison, p-value<0.05 marked for statistical significance.

RESULTS

Effects of $AlCl_3$ on lipid profile parameters and Cardiovascular Risk Indices

Data illustrated in table 1 show the mean values in a dose-response effect of $AlCl_3$ toxicity on the traditional lipid profile parameters and atherogenic indices in male rats for 28 days. It showed that $AlCl_3$ caused significant differences ($p<0.05$) in the damaged lipid profile markers in dose- dependently elevated TG (60.1%), TC (62.5%) and LDL-c (237.9%) and concomitantly concentration dependent significantly decrease ($p<0.05$) in low level of HDL-c (-84.9%) in 800mg/kg $AlCl_3$ when compared with the control group (fig 1).

Table 1 also shows that $AlCl_3$ induced state of dyslipidaemia (abnormal lipid profile) resulted to statistically significantly ($P<0.05$) alterations in all the indices of atherogenicity differentially in a dose-dependent manner in comparison with control. $AlCl_3$ perturbation of the atherogenic indices resulted in prevalence of AIP, CRI-1, CRI-11, and AC, increasing from the control values of 9.7, 5.1, 5.8 and 2.9% to 34, 54.8, 59.1 and 60.1% respectively in 800mg/kg $AlCl_3$ (fig 1).

Effects of $AlCl_3$ on the ratio of lipid profile

Table 2 shows the mean values of the ratio of lipid profile. $AlCl_3$ in a dose-response manner significantly increased differentially TG/HDL-c, TC/HDL-c, LDL-c/HDL-c, LDL-c/TG, and LDL-c/TC, TC-HDL-c/HDL-c ratios whilst a decrease in HDL-C/LDL-c ratio, from the control levels of 256.0, 93.4 , 193.7, 42.9, 22.1, 16.7 and 234.0% to 2706.8, 1977.6 1883.9, 913.6, 23.9, 18.6, and 10.5% respectively in 800mg/kg $AlCl_3$. Figure 2 shows the percentage relationship perturbation of $AlCl_3$ on lipid ratios in 800mg/kg $AlCl_3$ relative to control group.

Predictor ratio of AlCl3 induced perturbation of lipid profile and cardiac damaged biomarkers

Table 3 depicts predictor ratios for $AlCl_3$ induced alterations in lipid profile and cardiac damage biomarkers respectively. Table 3 illustrated that $AlCl_3$ toxicity lead to alterations in the indices of atherogenicity and ranked the predictor ratio generally as: AIP: CRI-11: AC: CRI-1. The predictor ratio revealed that atherogenicity indices - CRI-11, AC and CRI-1 increased from the control values 6.3, 6.3 and 12.8% to 43.8, 43.8 and 38.5% in 800mg/kg $AlCl_3$ respectively. Predictor ratio found no difference regarding AIP between the groups

Table 3 also depicted predictor ratio of abnormal lipid profiles and showed significant differences ($p<0.05$) among the groups. The predictor ratio for $AlCl_3$ damaged lipid profile at the concentration 0 and 200mg/kg were ranked as: LDL-c: HDL-c: TC: TG compared to $AlCl_3$ concentrations in excess of the order of 200mg/kg as: HDL-c: LDL-c: TC: TG. Predictor ratio revealed elevation of TG, TC and LDL-c from the control values of 11.5, 12.2 and 6.3% to 52, 51.2 and 62.5% and a decrease in HDL-c level from the control value of 33.3% to 16.7% in 800 mg/kg $AlCl_3$ respectively.

DISCUSSION

Our study expanded beyond the scope of the use of the traditional lipid profiles (total cholesterol (TC), LDL-cholesterol (LDL-c), triglycerides (TG) and HDL-cholesterol (HDL-c)) to probable atherogenic risk predictors such as Castelli's risk I & II (CRI = I & II) index, Atherogenic Coefficient (AC) and Atherogenic index of plasma (AIP) and lipid ratios in the assessment of the development of cardiovascular disorder in male wistar rats exposure to graded doses of aluminum chloride-tainted drinking water ($AlCl_3$) for 28 days. The findings of this study strengthens the reports elsewhere that $AlCl_3$ induces state of dyslipidaemia with atherogenic lipid profile with structural changes in cardiovascular systems and calling policy makers for timely intervention in terms of creating awareness, early detection and management. Our findings showed that $AlCl_3$ in a concentration dependent manner caused significantly ($p<0.05$) higher risk of developing cardiovascular diseases significantly differences in all estimated atherogenic indices, lipid profiles and lipid ratios comparing in both $AlCl_3$ and control groups. Our findings are suggestive that prolonged exposure to $AlCl_3$ the higher the risk of development of cardiovascular disease in agreement with time-dependent [22] adverse toxic effects of aluminium chloride on the heart.

Our present study revealed that the estimated atherogenic indices parameters showed an increased rate for $AlCl_3$ induced risk for cardiovascular disease and ranked as: Castelli's risk II (CRI=II)> atherogenic coefficient (AC) > Castelli's risk I (CRI=I)> Atherogenic index of plasma (AIP). It confirmed that atherogenic indices can contribute to estimation of risk of cardio vascular disorders in rats exposed to $AlCl_3$. . More so, the predictor ratio also revealed that $AlCl_3$

Table 1 Mean value of lipid profile and atherogenic indices

	Lipid Profile (mmol/l)				Atherogenic indices			
Dose /mg/kg/ body weight	**TG**	**TC**	**LDL-c**	**HDL-c**	**AIP**	**CRI-1**	**CRI-II**	**AC**
control	169.02±0.68	127.86±3.75	28.30±2.69	66.02±0.61	0.41	1.94	0.43	0.94
200	193.36±1.46*	149.90±0.67*	45.90± 1.37*	44.06±1.47*	0.64*	3.40*	1.04*	2.40*
400	214.70±10.30*	164.24±0.34*	65.54±2.06^{x}	42.10±4.12*	0.71*	3.90*	1.56*	2.90*
600	235.82±10.12*	178.94±2.44*	81.22±1.89*	22.68±0.75*	1.02*	7.89*	3.58*	6.89*
800	270.68±4.29*	207.76±7.63*	95.62±1.53*	10.00±1.76*	1.43*	20.78*	9.56*	19.78*

*Values are presented in mean ± sem. n= 10. P ≤ 0.05 *means values are statistically significant when compared to the control*

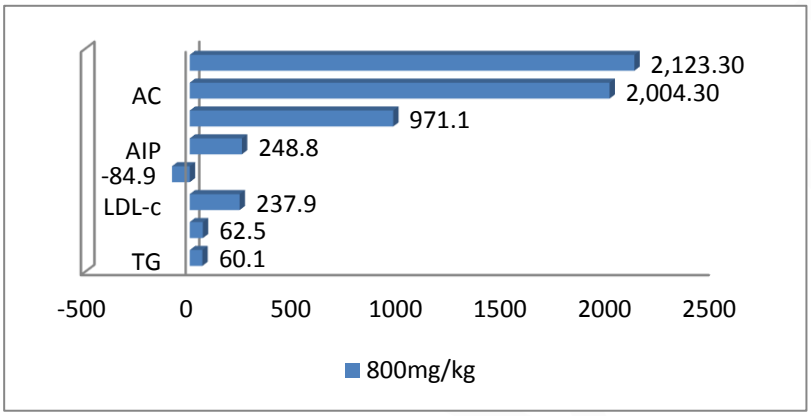

Figure 1 Percentage change in damaged lipid profile parameters and atherogenic indices relative to control

Table 2 Mean values of lipid ratio

Dose /mg/kg/ body weight	**TG/ HDL-c**	**TC /HDL-c**	**LDL-c /HDL-c**	**LDL-c /TC**	**LDL-c /TG**	**HDL-c /LDL-c**
control	256.0±1.12	193.7±6.15	42.9±4.41	22.1±0.72	16.7±4.00	234.0±0.23
200	438.9±1.00*	340.2±0.46*	104.2±1.00*	30.6±2.05*	23.7±0.93*	96.0±1.07*
400	511.0±2.50*	391.1±0.08*	155.7±0.50*	40.0±6.06*	30.5±0.20*	64.2±2.00*
600	1039.8±13.76*	789.0±2.25*	358.1±2.52*	45.4±0.78*	34.4±0.19*	28.0±0.40*
800	2706.8±2.44*	2077.6±4.33*	956.2±0.88*	46.0±0.20*	35.3±0.36*	10.5±1.15*

*Values are presented in mean ± sem. n= 10. P ≤ 0.05 *means values are statistically significant when compared to the control*

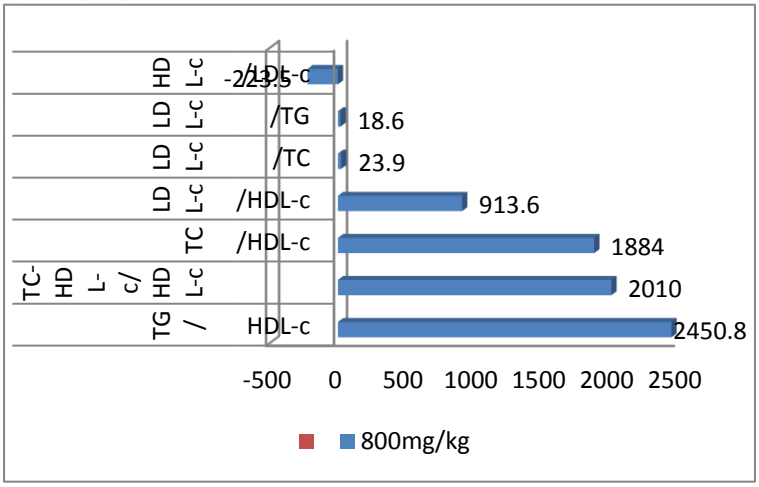

Figure 2 Percentage change in damaged lipid ratios relative to control

Table 4 Predictor ratio of $AlCl_3$ induced damaged tissue biomarkers

Dose/mg/kg/body weight	Ratio of association of lipid profile indices	Ratio of association of atherogenic indices
Control	LDL-c[1]:HDL-c[2]:TC[5]:TG[6]	AIP[1]: CRI-11[1]:AC[2]:CRI-1[5]
200	HDL-c [1]: LDL-c[1]:TC[3]:TG[4]	AIP[1]:CRI-11[2]:AC[4]:CRI-1[5]
400	HDL-c[1]:LDL-c[2]:TC[4]:TG[5]	AIP[1]:CRI-11[2]:AC[4]:CRI-1[6]
600	HDL-c[1]:LDL-c[2]:TC[8]:TG[10]	AIP[1]:CRI-11[4]:AC[8]:CRI-1[8]
800	HDL-c[1]:LDL-c[10]:TC[21]:TG[27]	AIP[1]:CRI-11[7]:AC[14]:CRI-1[15]

induced significantly differences in the cardiac damaged indices. Our findings observed is consistent with the univariable model [23] that support an increase in atherogenic index of plasma (AIP), defined as the logarithm of the ratio of plasma concentration of triglyceride (TG) to high-density lipoprotein cholesterol (HDL), recently proposed to be a novel marker in the identification of cardiovascular risk [17,18,24]. On the other hand, the predictor ratio model revealed that AIP may not be an independent factor that can impact the risk of cardiovascular disease in rats in agreement with multivariable model human studies [23].

Our study observed ranked lipid profile parameters frequently considered for prediction of vascular events as follows: Low density lipoprotein-cholesterol (LDL-c; 237.9%)>total cholesterol (TC, 62.5%)> triglyceride (TG; 60.1%) with low anti-atherogenic high density lipoprotein-cholesterol (HDL-c; -84.9%), in a dose-dependent manner, rose prevalence for CVD in 800mg/kg $AlCl_3$ compared with the control values. The lipid ratios that showed an increased rate for $AlCl_3$ induced risk for cardiovascular disease were, respectively, ranked as TG/HDL-c > (TC-HDL-c)/HDL-c >TC-HDL-c/HDL-c>TC/HDL-c > LDL-c/HDL-c, LDL-c/TC, LDL-c/TG with low HDL-c/LDL-c. But the lipid ratio parameters that showed strong attributable cardiovascular risks were, respectively, ranked: TG/HDL-c (182.9, 254.0, 784.7, and 2450%) followed by(TC-HDL-c)/HDL-c (156.1, 209.6, 635.6 and 2,010%) then TC/HDL-c (146, 196.4, 596.0, and 1883.9%), followed LDL-c/HDL-c (104.2, 155.7, 358.4 and 913.6%), and then LDL-c/TC (8.5, 17.9, 23.3, and 23.9%) followed by LDL-c/TG (7.0, 14.1, 17.7 and 18.6%) with low HDL-c/LDL-c (-138, -169.8, -206.0 and –223.5%) in 200, 400, 600 and 800mg/kg $AlCl_3$ respectively compared with control.

As observed in the present study and elsewhere it seems that the lipid parameter ratios included for the calculation of cardiovascular risks TG/HDL-c, TC-HDL-c/HDL-c, TC/HDL-c, LDL-c/HDL-c and HDL-c/LDL-c together with LDL-c and HDL-c are strong and independent risk factors for CVD in the experimental animals by $AlCl_3$. In a study with cardiovascular risk factors [5], HDL-c, TC/HDL-c and TG/HDL-c ratios has been attributable to higher risk for cardiovascular disease compared to other common biomarkers. However, the ratios TG/HDL-c and LDL-c/HDL-c were found to be stronger than TC/HDL-c, LDL-c/HDL-c and HDL-c as the most useful predictor of cardiovascular risk. In contrast, in their 21 day study [25] it was reported that TC, LDL-c levels, TC/HDL-c and LDL-c/HDL-c ratios were increased, while HDL-c and TG decreased in rats treated with AlCl3.

Interestingly, in order to further explore the relationship between lipid parameters and cardiovascular risk, the predictor ratio of $AlCl_3$ induced damaged lipid profile indices brought out the true picture about the overall attributable risks from total cholesterol (51.2%), low density lipoprotein (62.5%) and triglyceride (51.9%) whilst low high density lipoprotein (16.7%) compared to control values of 12.2%, 6.3%, 11.5% and 33.3% respectively.

Collectively, Castelli's risk index (CRI), atherogenic coefficient (AC) and ratios TG/HDL-c, (TC-HDL-c)/HDL-c, TC/HDL-c, and low HDL-C/LDL-c were found plausibly to determine approximately the severity of cardiovascular risk by $AlCl_3$ in rats compared with the values of lipid profile. Furthermore, our findings are in agreement with studies elsewhere that exposure to $AlCl_3$ could disturb lipid metabolism and alter normal blood lipid profiles in rats via direct inhibition of numerous enzymes related to lipid metabolisms.

Conclusion: the present study showed that oral administration of $AlCl_3$, for 28 days, dose-dependently elicited concomitantly proliferation of lipid profiles, lipid ratios and atherogenic indices differentially with resultant deleterious effect in cardiovascular cells and tissues in rats. Furthermore, the predictor ratio findings found to have high sensitivity and specificity for AIP showed no difference between the groups. Hence, calculating AIP may not be reliable in predicting the risk for development of cardiovascular risk in rats.

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