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Toxicological Assessment of Scratch-off Foils from Prepaid Cards in Male Wistar Rat

Fatoki JO, Adedosu OT, Adekunle AS, Afolabi OK, Badmus JA*

Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria

Abstract Evaluation of toxic effects of the scratch-off foil on pre-paid cards are important because of the fingernails scratching of the card's surface which lead to constant exposure to same by end users and retailers. Animals (n=18) were exposed to 10, 20 and 50 mg/kg body weight (b.w) foils from the cards three times a week for five weeks in corn oil through oral gavage while the control group (n=6) received corn oil only for the same period. Plasma and erythrocyte were analyzed for lipid dynamics and liver function tests. The results were analyzed using One-way ANOVA followed by Tukey's post hoc test. Hypercholesterolemia and phospholipidosis were the hallmark of the effects of the three doses of the foils, while hypertriglyceridemia characterized exposure to only 10 mg/kg b.w. Exposure to 10 and 20 mg/kg b.w. induced erythrocyte triacylglycerol constipation. Decreased high density lipoprotein cholesterol (HDL-C) and phospholipid concentrations with concomitant enrichment of HDL with triacylglycerol were observed at all doses tested compared to the control. Low density lipoprotein cholesterol (LDL-C) concentration was up regulated while triacylglycerol and phospholipid concentration was down regulated in all the three doses. Significant increase in plasma ALT, AST and γ-GT were observed in all the treated groups compared to the control. Exposure to 10 and 50 mg/kg b.w. caused 65% and 78% increase in ALT concentration. These findings revealed that foils from scratch-off cards up-/or down-regulate different pathways in the lipid metabolism spectrum and increased plasma activities of liver enzymes and therefore possess toxicological potential.

Keywords foils, hypercholesterolemia, hypertriglyceridemia, phospholipidosis, lipoproteins, liver enzymes

Introduction

It has become apparent that increasing human activities has modified the global cycle of heavy metals and metalloids [1, 2]. These heavy metals are ubiquitous in the environment as they abound in several environmental matrices such as water, plants, soil, air particulates, cosmetics as well as biological tissues [3-7] etc. Although, some of these metals are essential for human and at a very high concentration, they accumulate in body tissues and pose serious diverse health challenges [8].

Despite several scientific findings and reports, however, on the plethora of health effects associated with heavy metal, exposure to these metals continues unabated especially in the developing countries of the world. At present, one of the major routes of exposure of these elements is through the usage of various kinds of scratch-off cards [9], as it is very common to find particles of foils from these cards deposited under the fingernails and if hands are not properly washed, ingestion of these particles becomes easier alongside the food substance.

The retailers and users of pre-paid cards are exclusive of rural dwellers that used unwashed bared hands instead of cutlery for eating. The exposure to these categories of users who form the majority of Nigerian population could not be ruled out. Therefore, it is highly imperative to evaluatethe health implications of exposure to this foil. Scientific

findings have revealed heavy metal concentrations in the foils of these cards [9-12], to our knowledge, the effects of these foils of various scratch cards on lipid/ lipoprotein metabolisms and hepatic transaminases remain enigmatic. The present study, however, is aimed at exploring all these.

Materials and Methods

Test materials: Scratch-off cards from different GSM network providers such as Airtel, Glo, MTN, Etisalat and others from non-telecoms such as WAEC, NECO, JAMB etc. were collected from Ogbomoso and its environs. The foils were carefully scratched, ground into fine powdery form and transferred into a clean container. The following doses, 10, 20 and 50 mg/kg body weight of the test material were then prepared by weighing the appropriate quantity of the ground foils and were subsequently dissolved in corn oil which serves as a vehicle.

Chemicals: Cholesterol, triacylglycerol, AST, ALT and γ-GT kits are product of Fortress Diagnostics Limited, Northern Ireland, United Kingdom. All other reagents and chemicals are of analytical grade and were obtained from Sigma Chemical Co. St Louis, MO., USA.

Experimental animals: In an *in vivo* experimental study to examine the toxic effect of scratch-off foil from major pre-paid cards in Nigeria, twenty-four (24) male albino rats of the Wistar strain weighing between 120–150 g was purchased from the Animal House, Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria. The rats showed no visible signs of diseases or injuries. The rats were acclimatized for a week in the Animal House of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso and were fed with commercial rat pellets from Vita Feeds (Ibadan, Oyo State, Nigeria) and given water *ad libitum.* Animals were randomized into four experimental treatment groups of six rats each. Rats showing signs of lethargy or less than 120–150 g were excluded from the study.

Animal grouping and treatment: Group I serve as control and orally administered 1 ml of corn oil thrice weekly for five weeks, groups II, III and IV also dosed through oral gavage 10, 20 and 50 mg/kg body weight of the scratch-off foil three times each week for five weeks as shown below.

Group I: 1 ml corn oil (control)
Group II 10 mg/kg body weight
Group III 20 mg/kg body weight
Group IV 50 mg/kg body weight

At the end of the oral exposure period, blood was collected from the animals into heparinized tubes by cardiac puncture under light ether anesthesia after an overnight fast. Liver was removed from the animals for histological study. The blood samples were centrifuged at 5000 rpm for 10 min to separate plasma and red blood cells.

Biochemical analyses

Plasma and erythrocyte lipoprotein lipid profiles

Plasma and erythrocyte cholesterol and triacylglycerol concentrations were determined with commercial diagnostic kits obtained from Fortress Diagnostic, UK. High density lipoprotein (HDL) and low density lipoprotein + very low density lipoprotein (LDL + VLDL) cholesterol and triacylglycerol were also determined using the same commercial kits after LDL and VLDL were precipitated with heparin-$MnCl_2$ solution. Determination of Phospholipids in plasma, erythrocyte and lipoproteins followed the established procedure as described previously [13] after the phospholipids was extracted according to the method described elsewhere [14].

Enzyme assays

Plasma activities of ALT, AST andγ-GT were assayed using Fortress reagent kits (UK) according to the manufacturer's instructions as described previously [15, 16].

Statistical Analysis

Results were expressed as mean ± SD. The levels of homogeneity among the groups were assessed using Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Tukey's multiple comparison test. All analyses were done using GraphPad Prism® version 6.

Results

Tables depict the effects of the foils on plasma and erythrocyte (Table 1), HDL and LDL + VLDL (Table 2) lipid profiles of the animals. Exposure of the animals to the foils through oral gavage produced a plethora of dyslipidemic effects in these compartments. Hypercholesterolemia characterized the effects of the foils on the plasma of the exposed rats ($p<0.05$). Meanwhile, the doses of the foils had no significant effects on the plasma triacylglycerol level, while plasma phospholipid concentration was up-regulated by the foils at all doses but the increase was only significant at 10 mg/kg body weight.

Exposure to the foils resulted in a significant increase in cholesterol concentration ($p<0.05$) at all doses tested. The foils also increased the erythrocyte triacylglycerol content. The increase was sustained at only 10 and 20 mg/kg body weight and returned to control values at 50 mg/kg body weight. Similar to plasma phospholipid, the foils also up-regulated the erythrocyte phospholipid concentration, while the increase was also significant ($p<0.05$) only at 10 mg/kg body weight.

Following exposure to the three doses of the scratched off foils, there was about 50% decrease in HDL cholesterol and phospholipids contents (Table 2). Similarly, a significant reduction in HDL triacylglycerol contents was also observed following the exposure. In contrast to HDL phospholipids, LDL + VLDL phospholipids concentration (Table 2) was increased with all the doses of the foils with 10 mg/kg body weight producing the highest increase and 50 mg/kg body weight producing the least

The results of the hepatic transaminase evaluations in the plasma of rats exposed to the foils are presented in Table 3. Repeated oral doses of foils resulted in elevated plasma ALT, AST and ɣ-GT. The exposure resulted in a significant ($P < 0.001$) 4-fold dose dependent elevation of plasma ALT when compared with the control. Plasma level of γ-GT increased with foils treatment also in a dose dependent manner (IV > III > II) compared with the control group. On the contrary, the plasma AST showed reversed dose-dependent increase fashion with the highest dose inducing lowest increase activity.

Table 1: Effects of scratch-off foil from prepaid cards on Plasma and Erythrocyte lipid profile in Male Albino rats

	PLASMA			ERYTHROCYTE		
Group	**Cholesterol (mg/dl)**	**Triacylglycerol (mg/dl)**	**Phospholipids (mg/dl)**	**Cholesterol (mg/dl)**	**Triacylglycerol (mg/dl)**	**Phospholipids (mg/dl)**
I	87.58±3.87^{a}	49.44±5.34^{a}	74.08±8.20^{a}	101.00±8.16^{a}	19.06±1.13$^{a, b}$	135.30±4.28^{a}
II	107.50±2.45^{b}	52.12±5.94^{a}	102.20±8.97^{b}	179.80±11.85^{b}	21.04±1.91^{a}	234.90±3.13^{b}
III	91.55±5.62$^{a, d}$	47.75±1.72^{a}	83.83±3.41^{a}	190.30±8.01^{b}	20.70±0.84$^{a, b}$	128.60±14.11^{a}
IV	97.21±6.14$^{c, d}$	46.82±4.45^{a}	83.48±2.24^{a}	179.81±8.27^{b}	18.94±0.69^{b}	139.50±15.05^{a}

Results are presented as Mean±SD of six rats per group. SD =standard deviation, Mean values with different superscript letters are significantly different

Table 2: Effects of scratch-off foil from prepaid cards on HDL and LDL +VLDL lipid profile in Male Albino rats

	HDL			LDL+VLDL		
Group	**Cholesterol (mg/dl)**	**Triacylglycerol (mg/dl)**	**Phospholipids (mg/dl)**	**Cholesterol (mg/dl)**	**Triacylglycerol (mg/dl)**	**Phospholipids (mg/dl)**
I	53.50±6.49^{a}	24.31±1.95^{a}	67.83±6.91^{a}	2.67±0.83^{a}	3.49±0.84^{a}	36.65±6.51^{a}
II	28.50±0.87^{b}	21.10±2.28^{b}	33.18±7.60^{b}	6.34±0.08^{b}	5.64±0.31^{b}	46.78±6.80^{a}
III	21.50±1.82^{c}	21.96±1.06$^{a, b}$	36.06±3.48^{b}	5.14±0.66^{b}	7.43±0.70^{c}	46.66±6.40^{a}
IV	29.60±2.36^{b}	21.29±1.94^{b}	39.96±3.86^{b}	8.17±1.29^{c}	6.52±0.42$^{b, c}$	44.36±5.71^{a}

Results are presented as (Mean±SD) of six rats per group. SD =standard deviation, Mean values with different superscript letters are significantly different.

Table 3: Effects of scratch-off foil from prepaid cards on some biomarkers of Liver function in Male Albino rats

Groups	ALT (U/L)	AST (U/L)	ɣ-GT (U/L)
I	7.13±1.03[a]	95.07±3.62[a]	3.05±0.54[a]
II	7.69±0.28[a]	145.80±8.75[b]	4.71±0.20[b]
III	20.44±3.78[b]	128.40±3.28[c]	5.34±0.89[b]
IV	32.53±0.51[c]	118.10±0.67[d]	8.34±0.70[c]

Results are presented as (Mean±SD) of six rats per group. SD =standard deviation, Mean values with different superscript letters are significantly different.

Discussion

The perturbation of lipid metabolisms in various tissue compartments of the animals exposed to the scratched-off card foils in this study were characterized by up-/down dysregulation of cholesterol, triacylglycerol and phospholipids concentrations. Plasma lipid dynamics have been extensively studied in various metabolic malfunctions as a result of their involvement in cardiovascular disorders. Cholesterol and triacylglycerol are the components of foremost attention with very little recourse to plasma phospholipids as a biomarker in various pathologies.

The plasma phospholipid concentration was up-regulated at all doses of the foils, while triacylglycerol constipation was induced by 10 mg/kg body weight dose of the scratch card foils. Free fatty acids (FFA) are immediate substrates for phospholipid and triacylglycerol biosynthesis and also serve as the main energy source in most tissues [17]. Thus the elevated phospholipid and triacylglycerol concentrations in the treated groups imply that the absorbed FFA was more directed towards the synthesis of phospholipid and triacylglycerol. This can be linked to a possible suppression of fatty acid oxidation in the mitochondria with its attendance dire consequence of low energy yield. Metabolism of free fatty acid via β – oxidation pathway is the major source of energy in organs with high demand for energy [18, 19]. The abnormalities in erythrocyte lipid metabolism correlate with the etiology of several human diseases [20]. In addition to the difference between erythrocyte and plasma lipid compositions, the circulating mature erythrocytes in mammals are limited in their metabolism of lipid in two significant ways. Firstly, there is no substantial proof of *de novo* synthesis of lipid by the red blood cell [21, 22]. Secondly, the results obtained from both *in vivo* and *in vitro* studies suggest that a major pathway for replacement of red cell lipids is through exchange with plasma lipids [23-25]. Several compounds such as urea, acetone, alcohols and dimethylsulfoxide have the capacity to expedite this exchange [21]. Since the exposure to the scratch card foils led to elevated plasma concentrations of cholesterol and phospholipids at all concentrations and triacylglycerol at 10 mg/kg body weight, the increase observed in erythrocyte lipids may be attributed to the exchange of these lipids between plasma (lipoproteins) and red blood cells [24, 25]. For the maintenance of normal homeostasis, the organism most ensure a balance between the influx and efflux of metabolites in its diverse metabolic processes, an alteration in these levels might pose for the organism austere physiological consequences [26].

The diminished HDL phospholipids, that was observed following exposure to scratch cards foils may be as a result of an increased deposition and reduced degradation and transport of lipid from atherosclerotic lesions and thrombi and may therefore be a classical factor in the development of coronary artery disease [27]. Due to the amphiphilic properties of phospholipids, presence of phospholipid in HDL is an essential determinant of the capacity of HDL to remove and transport the non-polar cholesteryl esters, which contribute approximately 75% to HDL cholesterol (HDL–C) [27]. Therefore, reduced HDL –C levels are partly a consequence of low HDL phospholipids, as HDLs with reduced phospholipid contents have been found to be poor acceptors of cell cholesterol. Consequently, the severity of coronary artery disease has been found to be correlated strongly with a decrease in HDL phospholipids than with an increase in HDL cholesterol [27]. A decrease plasma level of HDL – C is a major risk factor for coronary artery disease [28] and it is most likely secondary to the increase hepatic secretion of triacylglycerol – rich lipoproteins [29]. This observed reduced HDL cholesterol concentration suggests that reverse cholesterol transport was inhibited by the exposure to the foils. Epidemiological studies have shown that increase concentrations of LDL

cholesterol in the blood are powerful risk factors for coronary heart disease [30, 31]. Exposure to the foils for five weeks resulted in increased plasma LDL cholesterol composition of the treated rats. Lipoprotein lipase usually acts on VLDL, converting it to LDL following the loss of triacylglycerol. This triacylglycerol loss is usually followed by an increase in cholesteryl ester, through the action of cholesteryl ester transfer protein (CETP) [32]. Cholesteryl ester is shuttled from HDL to apolipoprotein B-containing particles in exchange for triacylglycerol by CETP. The result of this exchange is the reduction of HDL cholesterol and elevation of non-HDL cholesterol [33].

Anti-inflammatory and antioxidant properties of HDL may also be modified by enhanced activity of CETP, thereby contributing to oxidative stress and chronic inflammation, which play a major role in the pathogenesis of oxidative stress related diseases [33-37]. Exposure to the scratch card foils resulted in a rapid elevation of the triacylglycerol concentration of LDL+VLDL, this is in relation to the decrease HDL cholesterol contents. It is, therefore, a pointer to the fact that the activity of CETP in its mediation of the exchange of triacylglycerol for cholesterol between non-HDL and HDL lipoprotein fraction has been compromised by the foils and hence the possible induction of oxidative stress.

Levels of certain enzymes such as hepatic transaminases (γ-GT, AST and ALT) are traditionally known to be indicators of hepatic functions/injury. Elevated plasma levels of these transaminases could be suggestive of an early liver dysfunction [38-41]. Our findings indicate that exposure of animals to scratch cards foils led to significant ($P<0.001$) increase in mean plasma γ-GT, AST and ALT when compared with the control group. These results imply hepatotoxicity effects of the foils of scratch cards. Remarkably, however, the plasma cholesterol, triacylglycerol, phospholipid, AST and erythrocyte phospholipids of 10 mg/kg foil group are significantly higher compared with higher concentration of scratch-off foil (20 and 50 mg/kg b.w). This implies that the scratch-off foil shows hormeticdose response influence as regard the levels of significant toxicity imparted by the 10 mg/kg treated group compared with higher doses (20 and 50 mg/kg b.w).

Several reports on the metal compositions of foils using Atomic Absorption spectrophotometer (AAS) have consistently implicatedPb, Cd, Ag, Cr, Ni, Cu and S as its constituents [9, 11, 12]. Bioaccumulations of these heavy metals in tissues are known to have very serious health concern as they are known to precipitate induction of oxidative related liver and kidney damage [42, 43]. Therefore, the toxicity of this scratch-off card foils as presented in this study might not be unconnected with the heavy metal constituents.

In conclusion, the findings of this study indicate that scratch-off card foils when ingested orally perturbs different pathways in the lipid metabolism spectrum and increased plasma activities of hepatic transaminases and therefore possess toxicological potential. Using fingernails to scratch off the foils therefore may not be a safe practice and should be discouraged. Regulatory agencies should ensure that warning statements concerning the toxicological potentials of these scratch-off card foils be clearly written on all scratch cards as a guide for potential users.

Author's Contributions

FJO, AOT, AAS, AOK and BJA participated in the conception, acquisition of data, statistical analysis and interpretation of results. FJO, AOT and BJA acquire the data and write the first draft of the manuscript. AAS, AOK and BJA supervised the statistical analysis, interpretation of data and revised the draft manuscript. All authors read and approved the final manuscript.

Author's Declaration

The authors declared no conflict of interest.

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