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Research Article
Disruption of Lipid Profile and Alteration of Hepatic Lipoprotein Metabolism Gene Expression in Anaemia-induced Rat

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Abstract

Background and Objective: Iron metabolism in animals is altered by haemolytic anaemia induced by phenylhydrazine (PHZ), however, its effects on lipid metabolism remains elusive. The aim of this study was to examine the impact of anaemia on lipid profiles and lipoprotein metabolism gene expression in rats. **Materials and Methods:** Fourteen adult male Wistar rats were randomly classified into normal control and anaemia-induced group (n = 7), respectively. Anaemia was induced in rats by daily administration of PHZ at 10 mg kg^{-1} for 8 consecutive days, after which blood was collected and liver excised. Lipid profiles of plasma and liver were determined spectrophotometrically while the expression of genes associated with lipid and lipoprotein metabolism was assayed by reverse transcriptase polymerase chain reaction. **Results:** The induced-anaemia resulted in hypotriglyceridemia and hypophospholipidosis, with concurrent hypercholesteromia compared to control, respectively. Liver triglycerides, phospholipids, cholesterol were observed to be up-regulated. Anaemic rats showed a significant ($p<0.05$) up-regulation of the relative expression of hepatic lecithin-cholesterol acyltransferase (Lcat), paraoxonase-1 (Pon-1), aryl hydrocarbon receptor (Ahr), 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr) and down-regulation of Scavenger Receptor Class B Type I (Scarb1). **Conclusion:** The induced-anaemia alter the expression of lipoprotein metabolizing genes which might be the underlying mechanism of anaemia to disrupt lipid metabolism.

Key words: Paraoxonase, anaemia, Phenylhydrazine, lecithin-cholesterol acyltransferase, metabolism

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anaemia, known as the reduction of oxygen carrying ability of the blood, is a common blood disorder affecting people of all ages and posing a great global healthcare[1,2]. It can be due to failure of red cell proliferation, defective maturation of red blood cells, blood loss and haemolysis[1,3]. Haemolytic anaemia is the accelerated destruction of mature red cells outside the bone marrow or a consequence of the destruction of imperfectly formed red cells[1,4]. Hemolytic conditions with substantial intravascular hemolysis include paroxysmal nocturnal hemoglobinuria (PNH), sickle-cell disease (SCD), thalassemias, hereditary spherocytosis and drug or chemical-induced anaemia[5,6]. The chemicals or drugs can caused haemolysis by interacting with sulfhydryl groups, inhibit various enzymes, disrupt immune mechanisms and fragment erythrocytes as they pass through the platelet-fibrin mesh[7]. Phenylhydrazine (PHZ) is an antipyretic drug that is well known for its ability to produce hemolysis in rats and humans leading to the formation of meta-haemoglobin which contributed to the oxidation of oxyhemoglobin[6-9]. Report shows that PHZ induced haemolytic anaemia in rats based on hepatic changes in the expression of genes that are mechanistically linked to haematotoxicity[7]. It has been suggested that PHZ induces haemolytic anaemia as a consequence of peroxidation of membrane lipids[10,11].

Cholesterol, triglycerides, phospholipids and fatty acids are lipids molecules that play key roles in metabolic pathways which are transported in the blood as lipoproteins[12-14]. Disturbances in the homeostasis of these lipids and lipoprotein resulted in dyslipidemia[15-19] which has effects on health, thus great attention is paid to abnormal levels of lipids and its associated factors[20-22]. The present study was undertaken to investigate the effect of PHZ-induced anaemia on the lipid profile and expression of lipoprotein metabolism genes in a rat model.

MATERIALS AND METHODS

Acclimatization of experimental animals: Ten weeks old inbred male albino rats (n = 14) weighing between 100 and 150 g were housed in clean cages. The rats were acclimatize to their environment for one week under standard 12 h light and dark cycles with free access to feed and clean tap water *ad libitum* before the experiment. The study was approved by the *ad hoc* Animal Ethical Committee of the Department of Biochemistry, Lagos State University, Ojo-Lagos, Nigeria and conducted in accordance with the ethical norms guiding principles of laboratory animal care[23].

Treatment protocol and tissue collection: The rats were randomly distributed into two groups of seven rats each. About 10 mg kg^{-1} b.wt., PHZ (Sigma-Aldrich Chemical Company, St Louis, MO, USA) was administered daily by oral gavage for 8 consecutive days[24] while the control rats were allowed free access to water. The rats were sacrificed on the 9th day, after an overnight fast, under ketamine anaesthesia and blood collected by cardiac puncture. Blood and liver were processed as previously described by Ogunrinola[25] and Rotimi *et al.*[26] while a portion of the left lobe was preserved in RNAlater®.

Biochemical analysis: Total plasma cholesterol and triglyceride concentrations were determined using commercially available kits according to the manufacturer's instructions. Plasma phospholipids was determined as described by Rifai *et al.*[27]. Lipids were extracted from the liver according to the method of Folch *et al.*[28] and the cholesterol, triglyceride and phospholipid determination was performed by the methods earlier described by Ogunrinola *et al.*[29] and Rotimi *et al.*[30], respectively.

Ribonucleic acid (RNA) extraction: The RNA was extracted from RNAlater®-stabilized liver using the Aidlab spin column RNA extraction kit according to the instructions of the manufacturer. Concentration and purity of extracted RNA was determined at 260 and 280 nm using a NanoDrop® 2000 spectrophotometer (Thermo Scientific). The RNA samples were kept at -80°C until gene expression analysis.

Expression of hepatic lipid metabolizing genes: The levels of expression of five lipid metabolizing genes (lecithin-cholesterol acyltransferase (Lcat), paraoxonase-1 (Pon-1), aryl hydrocarbon receptor (Ahr), 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr) and scavenger receptor class B Type I (Scarb1)) were assessed in the liver using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) as previously described by Rotimi *et al.*[31] using gene specific primers (GSP), which are designed based on known sequences of the target RNA (Table 1). The intensity of the migrated RNAs bands was analysed using Image J software[32]. Results were presented as relative expression of each gene in comparison with a housekeeping (β-actin, Actb) gene (ratio of intensity of each gene to that of β-actin, Actb).

Statistical analysis: All statistical analyses were performed using IBM SPSS® version 20.0 statistical software (IBM Corp., Armonk, NY, USA). Data were expressed as

Table 1: Sequences of gene-specific primers

Gene sequence (5'-3')	Templates
Ahr	Forward: GGGCCAAGAGCTTCTTTGATG NM_OO13 08255_ 1
	Reverse: GCAAGTCCTGCCAGTCTCTGA
Hmgcr	Forward: TCACTUCCATCTACAT"TU NM 013134.2
	Reverse: GACCACTTGCTTCCATTA
Scarb1	Forward: GGCAAATTTGGCCTGTTCGT
	Reverse: I\TM 031 541. I
	CCACAGCAATGGCAGGACTA
Lcat	Forward: AACTGGCTGTGCTACCGAAA
	Reverse: NM_017024.2
	TAGGTCTTGCCAAAGCCAGG
Actb	Forward: GTCAGGTCATCACTATCGGCAAT NM_0311 44.3
	Reverse: AGAGGTCTTTACGGATGTCAACGT
Pon1	Forward: TGCTGGCTCACAAGATTCAC I\TM 032077.1
	Reverse: TCAAAGCTGAGGACCTTCAAT

Mean±SEM of five replicates. Analysis of variance (ANOVA) was carried out to test for level of homogeneity at $p<0.05$ among the groups.

RESULTS AND DISCUSSION

Lipid profiles results indicates that induced-anaemia with PHZ significantly increased plasma cholesterol (Fig. 1a) and down-regulate triglyceride (Fig. 1b) and phospholipid (Fig. 1c). However, the levels of hepatic cholesterol (Fig. 2a), triglyceride (Fig. 2b) and phospholipid (Fig. 2c) were significantly ($p<0.05$) increased in the anaemia-induced group compared to control group.

The expression of five genes was assessed in liver samples from all treatment groups. The relative expression of hepatic aryl hydrocarbon receptor (Ahr) (Fig. 3a), lecithin-cholesterol acyltransferase (Lcat) (Fig. 3b), paraoxonase-1 (Pon-1) (Fig. 3c) and 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr) (Fig. 3d) were significantly increased in anaemic-induced rats corresponding to 38.29, 5.26, 6.48 and 29.80%, respectively while hepatic scavenger receptor class B type I (Scarb1) (Fig. 3e) was significantly ($p = 0.05$) down-regulated.

Lipid and lipoprotein abnormalities have been shown to be the predictors for the metabolic disturbances, including dyslipidemia, hypertension, diabetes, cardiovascular disease and liver dysfunction[29,33]. Abnormal lipid homeostasis has been reported in haematological disorders such as sickle cell anaemia which alter membrane fluidity and function of red blood cell[34,35]. These finding of elevated plasma cholesterol concentrations was in agreement with that of

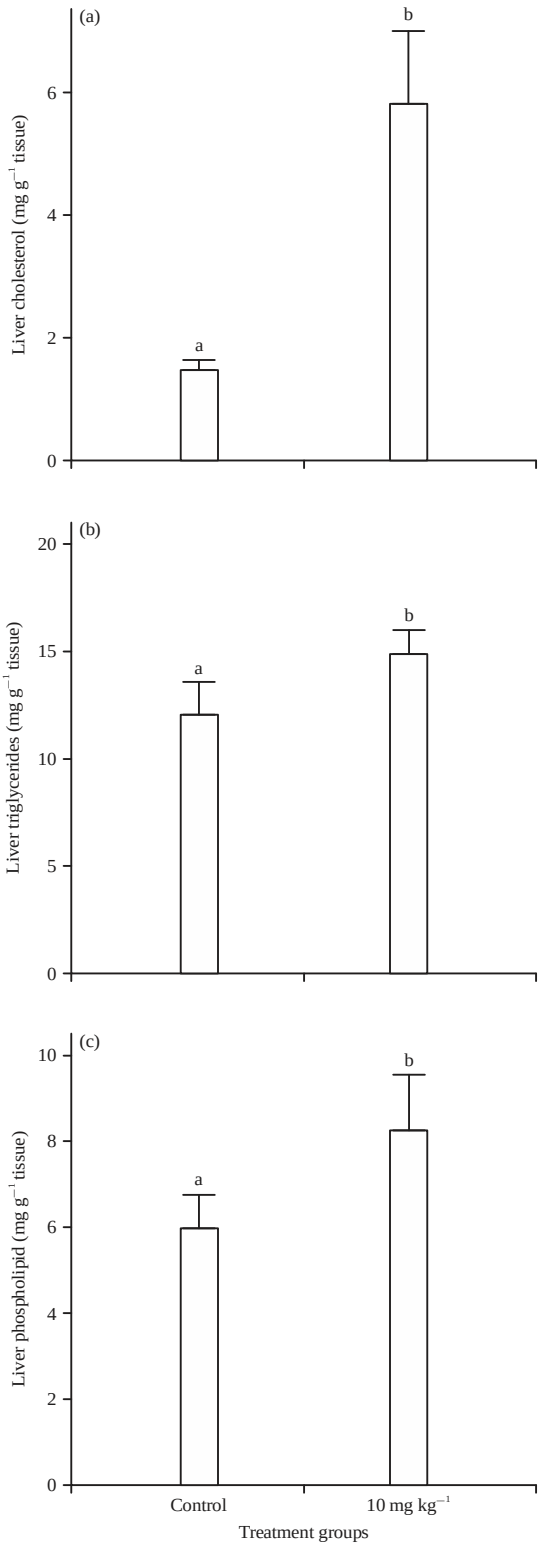

Fig. 1(a-c): Effects of PHZ induced-anaemia on plasma lipid profiles (a) Cholesterol, (b) Triglyceride and (c) Phospholipid

Bars represent the Means±SEM (n = 7). Bars with different alphabets are significantly ($p<0.05$) different from each other

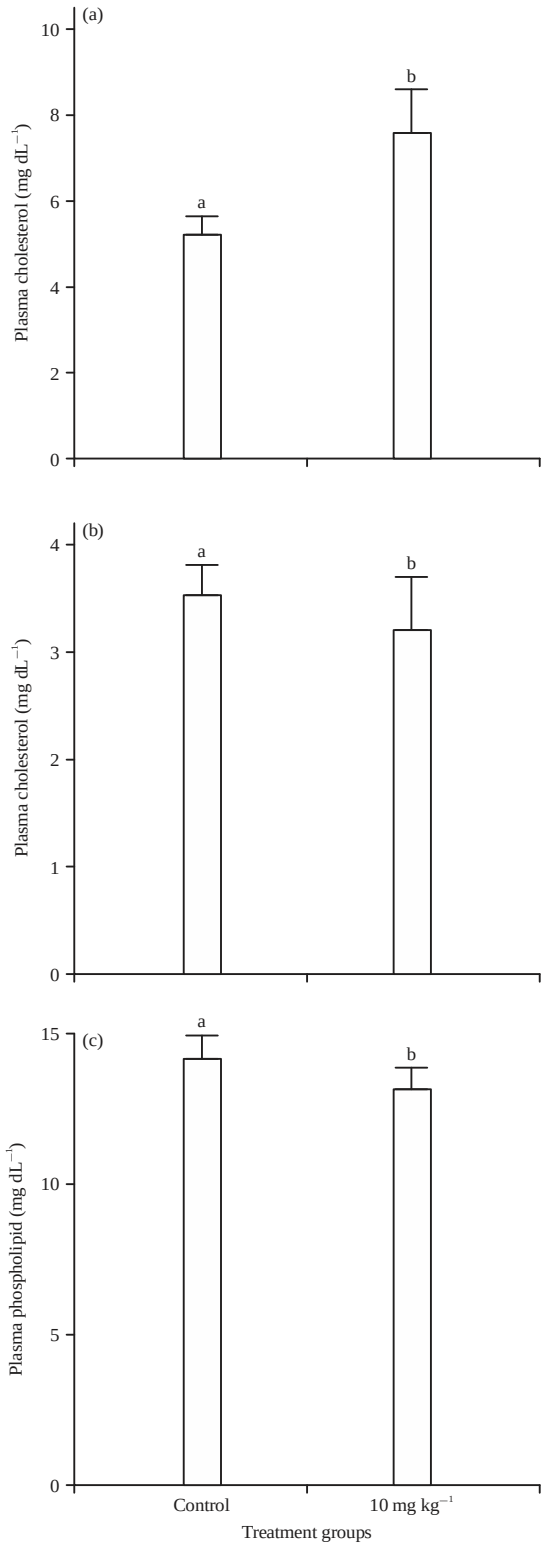

Fig. 2(a-c): Effect of PHZ induced-anaemia on hepatic lipid profiles (a) Cholesterol, (b) Triglycerides and (c) Phospholipid

Bars represent the Means±SEM (n = 7). Bars with different alphabets are significantly (p<0. 05) different from each other

Mazandarani and Hoseini[36]. This may be due to the concentration of red blood cell that is altered by cholesterol synthesis or its displacement from tissue to plasma[16] and can lead to abnormal cholesterol loading of the erythrocyte membrane in hemolytic anaemia patients with liver disease[37]. This study revealed hepatic accumulation of lipids which can either be due to excessive production and release of the lipids into circulation or by defective removal from the blood or the combination of both and could cause liver dysfunctioning[33,38]. The mechanisms underlying the observed accumulation of liver triglycerides may include increased liver fatty acid mobilization and delivery to the liver, increased hepatic lipogenesis and decreased secretion of very low density lipoprotein[39]. Also, the observed increase hepatic phospholipid may be due to heighten free fatty acid availability and/or increased cholesterogenesis[40,41].

Another finding of this study was that induced-anaemia elevated cholesterol concentration. This is in agreement with reports of Peng *et al.*[42] and some studies that there is relationship between hypercholesterolemia and anaemia having effect on atherosclerosis[16,43-45]. The up-regulation expression of hepatic 3-hydroxy-3-methylglutaryl-CoA Reductase (Hmgcr) gene contributed to the overall cholesterol status as observed in the present study. This is consistent with the reports of Ness and Chambers[46], Eisa-Beygi *et al.*[47] and Tian *et al.*[48]. Likewise, the present study showed that induced-anaemia resulted in activation of Lecithin cholesterol acyltransferase (Lcat) gene expression (important enzyme in the reverse cholesterol transport process)[49-51] which in turns elevated plasma cholesterol. The result also showed the synergistic effect of mRNA gene expression for Hmgcr and Lcat activation and increase in the level of plasma cholesterol in the induced anaemic rats.

The observed reduced gene expression of scavenger receptor class B Type I (Scarb 1) due to induced-anaemia in the rat may correlate with elevation of cholesterol levels. This result is consistent with report of Assanasen *et al.*[52] that the cholesterol flux mediated by Scarb1 plays a role in the regulation of signal-transduction initiation. In this present study the significant up-regulation of paraoxonase-1 (Pon-1) gene expression is in consistent with the report of Gong *et al.*[53]. The increase may be partially explained by the ability to reduce oxidative stress by scavenging oxidative free radicals and found to be associated with aryl hydrocarbon receptor (Ahr) dependent mechanism as supported by the report of Gouedard *et al.*[54]. The current study also revealed the elevation of Ahr gene expression in the liver which is similar to the report of Wada *et al.*[55]. This may be involve in

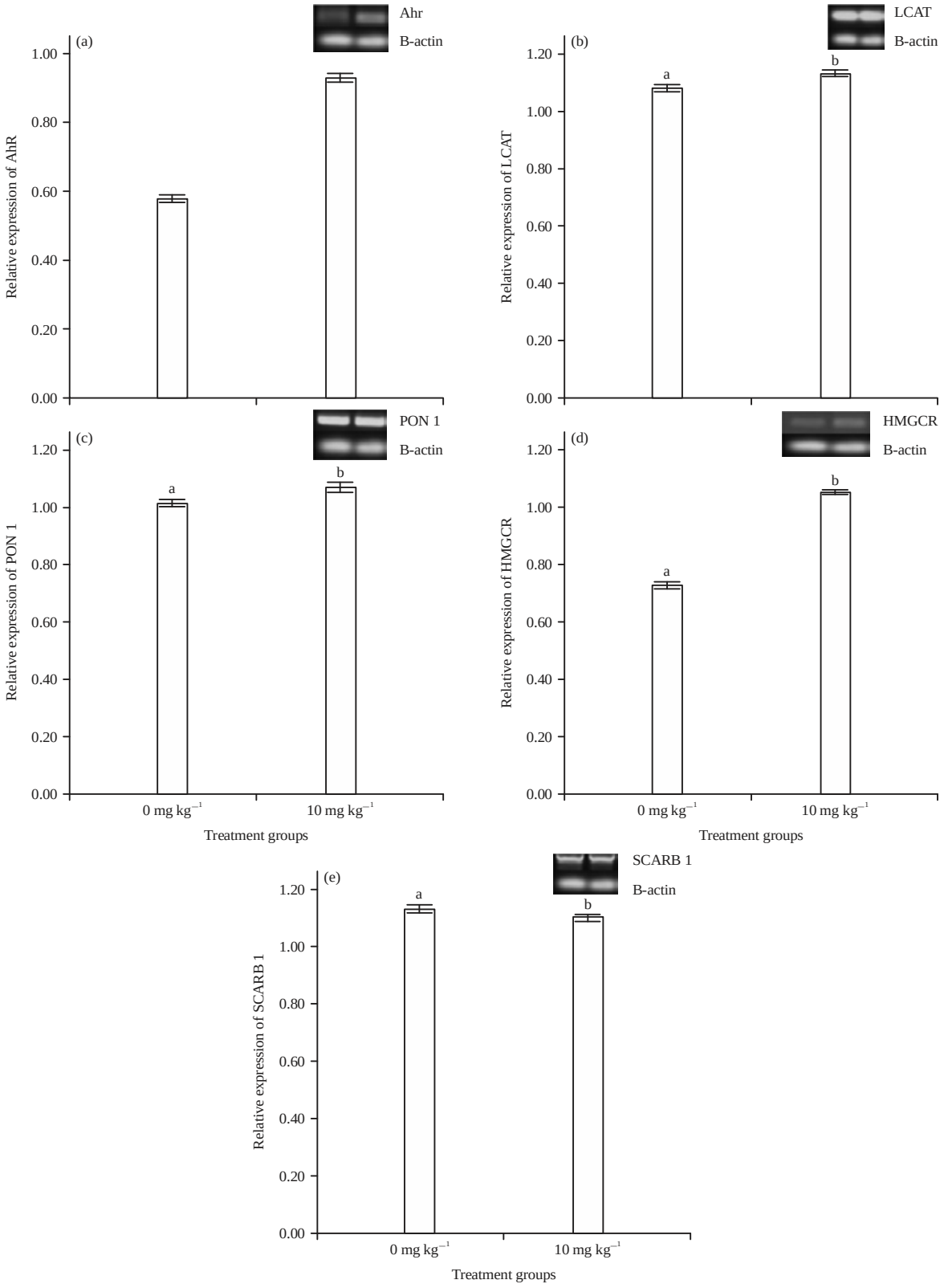

Fig. 3(a-e): Effects of induced-anaemia on relative expression of hepatic lipid metabolizing genes (a) Ahr, (b) Lcat, (c) Pon-1, (d) Hmgcr and (e) Scarb1

Bars represent the Means±SEM (n = 3). Relative expression is ratio of intensity of each gene to that of housekeeping gene (β-actin, Actb). Bars with different alphabets are significantly ($p<0.05$) different from each other

protecting the liver against lipotoxicity, transcription of cholesterol-biosynthetic genes-Hmgcr and elevated cholesterol, triglyceride and phospholipid through interaction with the transcription factor-sterol element binding protein 2, although this was not investigated.

CONCLUSION

Phenylhydrazine anaemic-induction in rat model for 8 days revealed hepatic dysfunction and dysregulation of lipid and lipoprotein metabolism through altered expression of Hmgcr, Pon1, Lcat, Scarb1 and Ahr genes. Notably, down-regulation of Scarb1 may be more sensitive indicator of anaemic-induction. Overall, the changes observed in this study may be associated to be the underlying mechanism whereby induced-anaemia signal liver damage.

SIGNIFICANCE STATEMENT

This study provides significant insight into the molecular changes sustained in expression of lipoprotein genes and lipid metabolism by PHZ induced-anaemia in a rat model. The mechanism of action of these changes may be due to the observed dyslipidemia, up regulation of liver lipids and dysregulation of lipoprotein metabolizing gene expression, which can ultimately be associated with the risk of cardiovascular diseases.

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