https://dx.doi.org/10.4314/jpb.v22i1.4
Vol. 22 no. 1, pp. 37-44 (January 2025)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Role of peroxisome proliferator-activated receptor (PPARα) in mediating renoprotective effects of fumarate

Osaze EDOSUYI[1,2*], Myung CHOI[2], Ighodaro IGBE[1], Adebayo OYEKAN[2]

[1]Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Benin City. Nigeria.
[2]Center for cardiovascular diseases, Gray Hall Suites, Rm 256, College of Pharmacy & Health Sciences Texas Southern University, 3100, Cleburne Street, Houston, Texas, USA.

Received 31st July 2024; Accepted 25th November 2024

Abstract
Fumarate has been reported to exert renoprotective actions. This study investigated a potential association between renoprotective activities of fumarate and peroxisome proliferator activated receptor (PPARα). PPARα wild type (WT) and knockout (KO) mice were randomly grouped into three–groups; control (mineral oil + distilled water, 10 mL/kg/day, p.o.), fumarate (1 mg/kg/day, p.o.) and fenofibrate (100 mg/kg/day, p.o.). Mice were treated for 10 days, after which they were anaesthetized with xylazine + ketamine, 100 mg/kg i.p. Cortical blood flow (CBF) was then measured using a laser Doppler. Biochemical analyses were carried out. Fumarate reduced CBF in PPARα WT mice compared to PPAR KO mice (280.3 ± 34.2 vs 449.8 ± 15 PU, $p<0.05$). Nitric oxide production was significantly lower in fumarate-treated PPAR WT compared to PPAR KO mice (13.6 ± 1 vs 20.9 ± 3 µM/ng, $p<0.05$). Catalase activity was insignificantly different in PPAR WT and KO mice treated with fumarate ($p>0.05$. Superoxide dismutase (SOD) activity was reduced by two-fold in PPAR WT mice compared to PPAR KO mice ($p<0.05$). This study demonstrates that the effect of fumarate on CBF, nitric oxide and L-arginine metabolism may be partially modulated through the PPARα downstream signalling pathway.

Keywords: Cortical blood flow; Fumarate; Nitric oxide; Peroxisome Proliferator-activated receptor (PPARα); Tricarboxylic acid cycle

INTRODUCTION

There is recent evidence that connects the intermediaries in the tricarboxylic acid (TCA) cycle to the aetiology of hypertension [1]. Fumarate, an intermediary in the TCA cycle, is central to this mechanism and has been shown to act as an antihypertensive with cardiorenal protective properties, [2, 3] exerting its action via an increase in nitric oxide production in the kidneys.

Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that regulate important cell processes such as lipid metabolism and inflammation in the cardiorenal system [4]. They exist as three [3] isoforms including, PPARα, PPARβ and PPARγ which are abundantly expressed in the kidneys and play prominent roles in renal control of blood pressure [5, 6]. The lack of PPARα (as seen in PPARα knockout (KO) mice) has been reported to led to a

*Correspondence. *E-mail*: Osaze.edosuyi@uniben.edu *Tel*: +234-8025228545 ISSN 0189-8442

compromised renal function as a result of the development of focal cortical necrosis. This highlights the prominent homeostatic role PPARα plays in the kidney [7] and the cardiorenal system. These receptors regulate vasomotor tone, inflammation, mitochondrial energetics, and energy metabolism [8-10]. PPARs play a significant role in understanding the complex relationship between inflammation, metabolism, and cardiorenal disorders especially as these sequelae underlie the aetiology of cardiorenal disorders [11]. Several studies have reported on the interactions between PPAR and other signalling systems. In one of such studies, clofibrate, the PPARα agonist, reduced blood pressure in salt-sensitive rats via an interaction with cytochrome P450 enzymes in the proximal tubules of the kidneys [12]. Similarly. Fenofibrate has been reported to improve renal haemodynamics in renal failure [13].

PPARs have also demonstrated the ability to regulate the expression and transcription of genes that modulate cardiorenal pathologies. PPARγ activation has been shown to inhibit vascular smooth muscle cell (VSMC) proliferation and expression of inducible nitric oxide synthase (iNOS) [14]. Furthermore, the association between PPAR and the cardiorenal system is supported by the presence of PPARα and PPARγ receptors in the endothelium especially as endothelial integrity is central to maintaining cardiorenal homeostasis. Experimental evidence exists, indicating that PPAR activation is beneficial in hypertension while studies have reported that PPARγ activation blunted angiotensin (Ang) II-induced hypertension and pathological changes [15]. Similarly, PPARγ agonist, blunted deoxycorticosterone acetate (DOCA)-salt-induced endothelial dysfunction [8]. The cardiorenal protective effects of PPARs appear to be linked to the modulation of nitric oxide production.

Given the role of PPARα in the cardiorenal system, this study explores the potential mechanistic interactions between fumarate and PPARα downstream signalling pathways.

EXPERIMENTAL METHODS

Animals. Thirty-six (36) mice (18 PPARα WT and 18 PPARα KO) (C57BL/6 strain) (18 – 35 g) were purchased from Jackson Laboratory (Bar Harbor, ME) and were inbred in the animal facility of Texas Southern University, Houston, Texas, USA. The animals were housed in clean cages, under a 12-hour lightening cycle, and provided with standard mice food (Purina Chow; Purina, St Louis MO, USA). The mice had free access to water and food throughout the study period. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Texas Southern University, Houston, Texas. This work was carried out in adherence to the Care and Use of Laboratory Animals guidelines of the National Institutes of Health [29].

Experimental protocol. PPAR wild-type (WT) and Knockout (KO) mice were randomly divided into three (3) groups, each containing six (6) mice each; PPAR WT (n=6) and PPAR KO (n=6). Group I: control (mineral oil + distilled water, 10 mL/kg/day, *p.o.*), group II: fumarate (1 mg/kg/day, *p.o.*) and group III: Fenofibrate, suspended in mineral oil, (100 mg/kg/day, *p.o.*). Mice in each group were treated for 10 days. After 10 days, mice were anesthetized with ketamine + xylazine (100 mg/kg, *i.p.*) and a left-sided laparotomy was performed to expose the left kidney. Cortical blood flow (CBF) was measured by placing a laser Doppler probe on the surface of the kidney. The kidneys were then excised, homogenized in lysate buffer, and stored at -80°C for biochemical studies.

Homogenization of excised kidneys. The homogenization of the excised kidney was

performed according to the protocol in the Thermo Scientific® NE-PER nuclear and cytoplasmic extraction reagents kit. Phosphate buffered saline (PBS, 1 mL, pH 7.4) was added to the kidney (180 ± 0.2 mg). The kidney/PBS mixture was then centrifuged for 5 minutes at 500 g to remove possible debris. The homogenizing mixture was then constituted by mixing 3 mL of cytoplasmic extraction reagent I (CER I), 0.165 mL of cytoplasmic extraction reagent II (CER II), and 0.15 mL of nuclear extraction reagent (NER). This homogenization mixture was then added to the kidney/PBS mixture and the resulting mixture was homogenized using a homogenizer [16].

Protein concentration assay to determine renal protein content. The Thermo Scientific® micro-BCA protein assay kit procedure was applied in this assay. Briefly, 25 parts MA + 24 parts MB + 0.5 parts MC was mixed to prepare the BCA reagent. This was by the addition of 0.04 mL of the homogenized kidney fraction to 0.5 mL of BCA working reagent and 0.497 mL of distilled water. The mixture was kept at 60℃ in a hot-water bath for 1 hour and read spectrophotometrically at 562 nm. Standard albumin concentrations (4, 8, 10, 12, 16, and 20 µg/mL) were also prepared and read against a reagent blank (0.03 mL of sample mixture, 0.5 mL of BCA working reagent, and 0.497 of distilled water).

Renal nitric oxide quantification assay. Briefly, Solution A (1 g sulphanilamide in 5 mL of phosphoric acid and 95 mL of distilled water) and Solution B (100 mg of N-(-1-naphthyl) ethylenediamine (NEDD) in 100 mL of distilled water) were mixed in a 1:1 ratio to prepare the Griess reagent. This was followed by the addition of 0.5 mL of the kidney homogenate to 0.5 mL of Griess reagent. Standard concentrations of sodium nitrite ($NaNO_2$) (1, 2, 5, 10, and 20 µM) were prepared and 0.5 mL of Griess reagent was added to each standard concentration. All mixtures were read immediately at 540 nm against a reagent blank [17].

Renal catalase assay. This assay was based on the spectrophotometric determination of the amount of hydrogen peroxide (H_2O_2) degraded per time. Briefly, 40 µg of the kidney homogenate was added to 0.48 mL of Phosphate buffer and 0.50 µL of 20 mM H_2O_2. The resulting mixture was immediately read at 240 nm, every 30 s for 210 s, against a reagent blank. Catalase activity was estimated as described [18].

Renal superoxide dismutase assay. This spectrophotometric procedure was based on the inhibition of the autooxidation of epinephrine by superoxide dismutase. Briefly, 40 µg of the kidney homogenate was mixed with 0.5 mL of 0.05M carbonate buffer (pH 10.2) and 0.5 mL of 0.3 mM adrenalin. The resulting mixture was read at 480nm for 180 seconds at intervals of 30 seconds against a reagent blank. Superoxide dismutase activity was deduced as described by Misra and Fridovich [19].

Renal arginase assay. This procedure involved the spectrophotometric measure of ornithine as an indirect measurement of arginase activity. Briefly, 40 µg of kidney homogenate was incubated with 1 M of magnesium chloride ($MgCl_2$) for 30 minutes at 37℃. This was followed by the addition of 0.5 mL of L-arginine buffer to the mixture with incubation for 1 hour at 37℃. After 1 hour, the developer solution containing, 0.05 mL of ninhydrin + 0.45 mL of acetic acid + 0.05 mL of phosphoric acid solution with incubation for 1 hour at 95℃. Standard concentrations of ornithine (1, 5, 10, 25, 50, and 100 µM) were prepared and read against a blank solution at 530 nm [20].

Statistical analysis. Statistical analysis was carried out using GraphPad prism® 6.0 software. Results were presented as mean±standard error of mean (SEM). One-way analysis of variance (ANOVA) was used for comparisons within and between PPAR WT and KO mice groups, followed by Tukey's

post hoc test. $p<0.05$ was considered significant.

RESULTS

Effect of fumarate on cortical blood flow (CBF) in PPAR WT and KO mice. As illustrated in Figure 1, fenofibrate significantly increased CBF in PPARα WT and KO mice, when compared to control ($p<0.05$). Fumarate showed an insignificant effect on CBF in PPARα WT mice compared to PPARα WT control mice ($p>0.05$) but induced an increase in CBF in PPARα KO mice when compared to PPARα KO mice ($p < 0.05$). The increase in CBF in fumarate-treated PPAR KO mice was significant when compared to fumarate-treated WT mice ($p<0.05$).

Effect of fumarate on nitric oxide production in PPARα wildtype and knockout mice. An increase in nitric oxide production in the kidneys of fenofibrate-treated PPARα WT mice was observed as depicted in Figure 2 when compared to PPARα WT control mice ($p<0.05$). Administration of fumarate did not cause any change in nitric oxide production in PPARα WT mice but increased the NO production in PPARα KO mice, compared to PPARα WT and KO controls respectively. (13.6 ± 1 vs 20.9 ± 3 μM/ng, $p<0.05$).

Effect of fumarate on arginase activity in PPAR WT and KO mice. The effect of fumarate on arginase activity was evaluated for any possible interaction with PPARα. As illustrated in Figure 3, fenofibrate, a PPARα agonist tended to reduce arginase activity in PPARα WT when compared to PPARα WT control mice ($p > 0.05$) but produced an 11-fold increase in arginase activity in PPARα KO mice ($p < 0.05$). Fumarate elicited no change in arginase activity in PPARα WT mice when compared to PPARα WT control mice ($p > 0.05$, n=5). However, fumarate increased arginase activity (14-fold, $p < 0.05$) in PPARα KO mice when compared to PPARα WT mice.

Catalase and SOD activities in PPAR WT and KO mice treated with fumarate. Figure 4a shows that fenofibrate reduced SOD activity in PPARα KO mice when compared to PPARα KO control ($p<0.05$) and increased SOD activity in PPARα WT mice, when compared to PPARα WT control mice (45 %, $p>0.05$). On the contrary, fumarate did not have any significant effect on SOD activity in PPARα KO and WT mice, when compared to the respective controls ($p<0.05$). However, SOD activity in fumarate-treated PPARα WT mice was significantly reduced (2-fold, $p<0.05$) compared to fumarate-treated PPARα KO mice. As illustrated in Figure 4b, fenofibrate and fumarate evoked a time-dependent increase in catalase activity in PPARα WT and KO mice, compared to the respective controls ($p<0.05$).

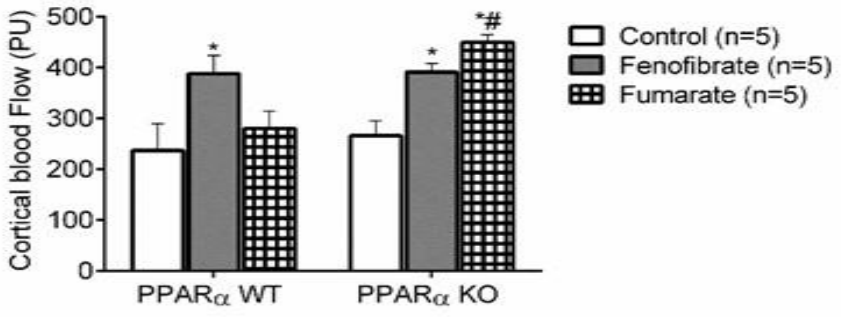

Figure 1: Effect of fumarate (1 mg/kg/day, *p.o*) or fenofibrate (100 mg/kg/day, *p.o*) on cortical blood flow (CBF) in peroxisome proliferator activated receptors (PPARα) wild-type (WT) and knockout (KO) mice after 10 days. *$p<0.05$ compared to control; #$p<0.05$ compared to fumarate-treated PPARα WT mice

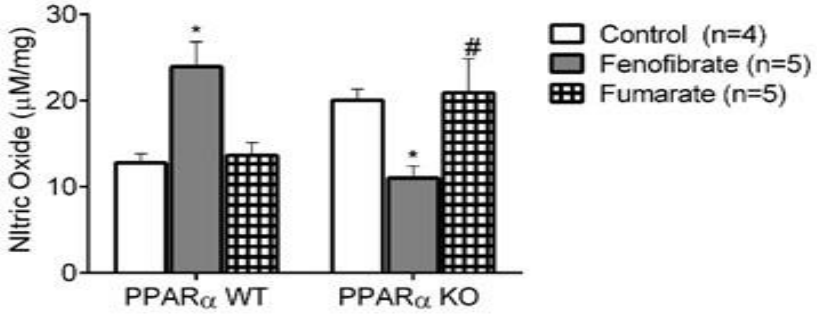

Figure 2: Effect of fumarate on nitric oxide production in PPARα WT and KO mice. Nitric oxide produced in the kidneys of PPARα WT and PPARα KO mice treated with fumarate (1 mg/kg/day, *p.o*) or fenofibrate (100 mg/kg/day, *p.o*) for 10 days. *p<0.05 compared to control. #p<0.05 compared to fumarate-treated PPARα WT mice.

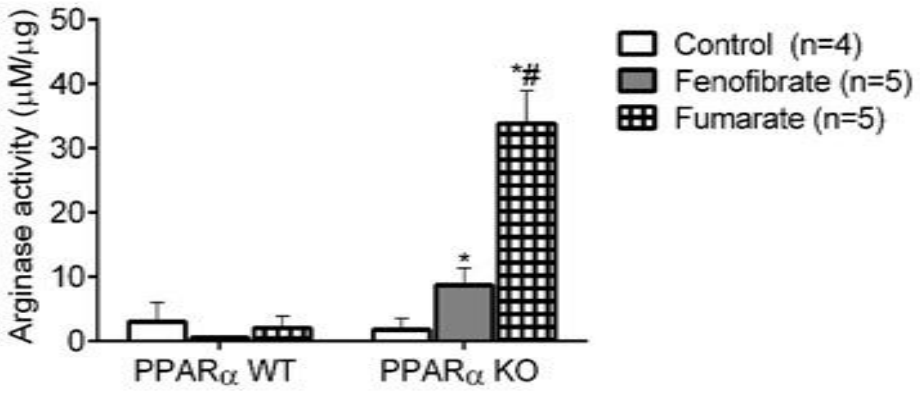

Figure 3: Renal arginase activity in PPARα WT and KO mice. Renal arginase activity in PPARα WT and PPARα KO mice treated with fumarate (1 mg/kg/day, *p.o*) or fenofibrate (100 mg/kg/day, *p.o*) for 10 days. *p<0.05 compared to control. #p<0.05 compared to fumarate-treated PPARα WT mice.

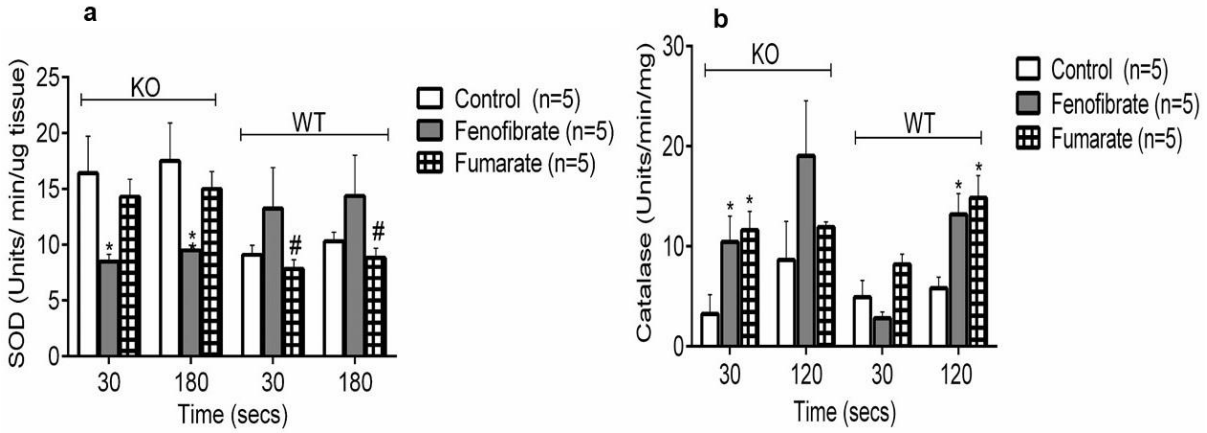

Figure 4: Effect of fumarate on superoxide dismutase and catalase activities in PPARα WT or KO mice. Effect of fumarate (1 mg/kg/day, *p.o*) or fenofibrate (100 mg/kg/day, *p.o*) on superoxide dismutase (SOD) and catalase (CAT) activities in peroxisome proliferator activated receptors (PPARα) wild-type (WT) and knockout (KO) mice after 10 days. *p<0.05 compared to control. #p<0.05 compared to fumarate-treated PPARα KO mice.

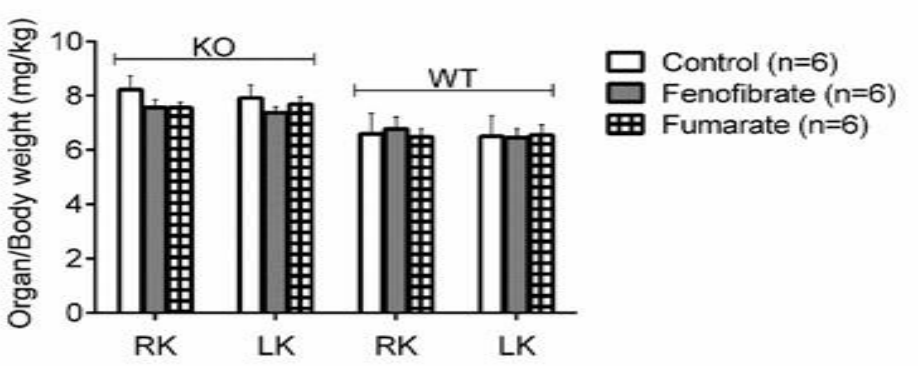

Figure 5: Kidney/body weight ratio of PPARα WT and PPARα KO mice treated with fumarate (1 mg/kg/day, *p.o*) or fenofibrate (100 mg/kg/day, *p.o*) for 10 days. RK=right kidney. LK=left kidney.

There was no significant difference in catalase activity in both PPAR WT and KO treated with fumarate ($p>0.05$).

Effect of fumarate on organ weights in PPAR WT and KO mice. Organ weights as an index of hypertrophy were evaluated in PPARα WT and KO mice treated with fumarate. As illustrated in Figure 5, kidney weights in fenofibrate and fumarate-treated PPARα WT and KO mice were not significantly different from PPARα WT and KO control mice ($p>0.05$, $n=5$). However, kidney weights were larger in PPARα KO mice when compared to PPARα WT mice.

DISCUSSION

Peroxisome proliferator activated receptors (PPARs) in the kidneys play vital roles in maintaining homeostasis and regulating the expression of genes [21]. For instance, activation of PPARα has been shown to enhance the expression of nitric oxide synthase (NOS) [22, 23]. To investigate the potential mechanistic link between PPARα and effects of fumarate, kidneys from PPARα wild-type (WT) and knockout (KO) mice were evaluated. Fenofibrate, a PPARα agonist increased catalase activity in PPARα WT mice but not in KO mice, highlighting the relationship between PPARα and catalase activity. This relationship has been corroborated by several studies showing that PPAR response elements (PPRE) are located in the promoter regions of the catalase gene [24].

Fumarate increased the activities of CAT and SOD In the absence of PPARα indicating that its effect on these enzymes was not dependent on PPARα downstream signalling. Similarly, fumarate increased NO production in KO mice suggesting a regulatory effect of PPARα on the renal effects of fumarate as it relates to the redox state and NO production. This may be connected to the interaction between PPARα and glucose metabolism; PPARα has been reported to downregulate glucokinase and pyruvate kinase, two key enzymes necessary to generate pyruvate needed in the TCA cycle [25, 26]. Hence, in PPARα WT mice, fumarate production is blunted and by extension, its anti-oxidant and NO activity. On the contrary, the absence of PPARα supposedly enhances the effect of fumarate via the increased glucokinase and pyruvate activities. This may partly suggest why fumarate tended to exert a reduced effect in PPARα WT but not KO mice as it concerns SOD activity and NO production.

Arginase impacts nitric oxide (NO) production by decreasing the availability of L-arginine. In this study, arginase activity was reduced in fenofibrate and fumarate-treated PPARα WT mice as expected while opposite effects were observed in PPARα KO mice.

Previous experimental evidence has shown a connection between PPARs and arginase activity. For example, a 2008 study by Gallardo-Soler and colleagues, reported that low-density lipoproteins (LDL) increased arginase activity in macrophages through interaction with PPAR γ/δ [27]. Similarly, data from this study showed an interaction between PPARα and arginase activity. Hence, fumarate may partly reduce arginase activity via PPARα-induced suppression of arginase activity. This may underpin the increase in NO production observed in fenofibrate and fumarate-treated PPARα WT mice. It seems likely that PPARα activation counteracts the effects of fumarate which may be necessary to prevent an 'overdrive' of antioxidant and vasodilatory effects caused by an unchecked increase in superoxide dismutase activity and nitric oxide production. Similarly, cortical blood flow was reduced in fumarate-treated PPAR wildtype mice but was increased more significantly in PPARα KO mice. This suggests that fumarate's impact on cortical blood flow may be partially linked to PPAR-α activation. Cortical blood flow is a tightly regulated process involving the participation of numerous target genes and mediators, including PPARα. In the absence of PPARα activation, other genes may be upregulated to maintain renal perfusion and prevent hypoxia. This may explain the lack of significant differences in cortical blood flow in PPAR WT and KO mice treated with fenofibrate.

PPARs are highly expressed in the kidneys, especially in the glomeruli and tubules where they stimulate lipolysis and prevent lipid build-up [28]; a process that is inhibited in PPARα KO mice due to the absence of PPARα and this may partly underline the larger organs in the PPARα KO mice.

Conclusion. This study demonstrates that PPARα plays a significant role in modulating the renal effects of fumarate. The study further emphasizes that intermediaries in the TCA cycle, which are downstream of fumarate have connections with other regulatory pathways including PPARα within the renal system.

Funding. This work was funded by a grant (5 G12 MD007605) from the National Institutes of Health.

REFERENCES

1. Zheng X, Tian Z. Fumarate hydratase as a potential target to ameliorate salt sensitive hypertension. Int. J Cardiol Cadiovasc Dis. 2022;2:14-16.
2. Tian Z, Liang M. Renal metabolism and hypertension. Nature Comm. 2021;12:1-12.
3. Edosuyi O, Igbe I, Oyekan A. Fumarate and its downstream signalling pathways in the cardiorenal system: Recent insights and novel expositions in the etiology of hypertension. Eur J Pharmacol. 2023;961:1-6.
4. Guan Y, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): Novel therapeutic targets in renal disease. Kidney Int. 2001;60:14-30.
5. Hamblin M, Chang L, Fan Y, Zhang J, Chen YE. PPARs and the Cardiovascular System. Antioxid Redox Signal. 2009;11:1415-52.
6. Seiri P, Abi A, Soukhtanloo M. PPAR-γ: Its ligand and its regulation by microRNAs. J Cell Biochem. 2019;7:10893-10908.
7. Ezinne Ozurumba, Omana Mathew, Katsuri Ranganna MC and AO. Regulation of hypoxia inducible factor/prolyl and high salt diet hydroxylase binding domain proteins 1 by PPARα. J Basic Clin Physiol Pharmacol. 2017/2018;2:165-173.
8. Oyekan A. PPARs and their effects on the cardiovascular system. Clin Exp Hypertens. 2011;33:287-93.
9. Corona JC, Duchen MR. PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. Free Radic Biol Med. 2016;100:153-63.
10. Li S, Yang B, Du Y, Lin Y, Liu J, Huang S, et al. Targeting PPARα for the treatment and understanding of cardiovascular diseases. Cell Physiol Biochem. 2019;51:2760-75.
11. Yang J, Zhou Y, Guan Y. PPARγ as a therapeutic target in diabetic nephropathy and other renal diseases. Curr Opin Nephrol Hypertens. 2012;21:97-105.
12. Johnson EF, Palmer CNA, Griffin KJ, Hsu M. Role of the peroxisome proliferator-activated receptor in

cytochrome P450 4A gene regulation. The FASEB Journal. 1996;10:1241-8.

13. Adedapo AA, Oyekan AO. Effects of fenofibrate, a PPAR-α ligand, on the haemodynamics of glycerol-induced renal failure in rats. Hum Exp Toxicol. 2013;32:323–31.

14. Ricote M, Huang JT, Welch JS, Glass CK. The peroxisome proliferator-activated receptorγ (PPARγ) as a regulator of monocyte/macrophage function. J Leukoc Biol.1999:9;733–9.

15. Diep Q. PPARα activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats. J Mol Cell Cardiol. 2004;36:295–304.

16. Edosuyi O, Choi M, Edosuyi V, Igbe I, Oyekan A. Malate reduced kidney injury molecule (KIM-1) expression and selectively upregulated the renal nitric oxide production in obstructive nephropathy. Bratis Med J. 2023;124:151–7.

17. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS TS. Analysis of nitrate, nitrite, and [15N] nitrate and nitrate in biological fluids. Anal Biochem. 1982;126:131–8.

18. Aebi H. [13] Catalase in vitro. In: Methods in Enzymology. Academic Press; 1984. p. 121–6.

19. Misra HP, Fridovich I. The Role of Superoxide Anion in the Epinephrine and a Simple Assay for Superoxide Dismutase. J Biol Chem. 1972;247:3170–5.

20. Iyamu EW, Asakura T, Woods GM. A colorimetric microplate assay method for high-throughput analysis of arginase activity in vitro. Anal Biochem. 2008;383:332–4.

21. Jiang Y, Zhang Q. Catalpol ameliorates doxorubicin induced inflammation and oxidative stress in H9C2 cells through PPAR γ activation. Exp Ther Med. 2020;20:1003–11.

22. Leibovitz E, Schiffrin EL. PPAR activation: A new target for the treatment of hypertension. J Cardiovasc Pharmacol. 2007;50:120–5.

23. Bulhak AA, Sjöquist PO, Xu CB, Edvinsson L, Pernow J. Protection against myocardial ischaemia/reperfusion injury by PPAR-α activation is related to production of nitric oxide and endothelin-1. Basic Res Cardiol. 2006;101:244–52.

24. Girnun GD, Domann FE, Moore SA, Robbins MEC. Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter. J Mol Endocrinol. 2002;16:2793–801.

25. Xu J, Xiao G, Tirujillo C, Chang V, Blanco L, Joseph SB, et al. Peroxisome proliferator-activated receptor α (PPARα) influences: Substrate utilization for hepatic glucose production. J Biol Chem. 2002;277:50237–44.

26. Peters A, Baes M. Role of PPAR in Hepatic Carbohydrate Metabolism. PPAR Res. 2010;2010:1–12.

27. Gallardo-Soler A, Gómez-Nieto C, Campo ML, Marathe C, Tontonoz P, Castrillo A, et al. Arginase I induction by modified lipoproteins in macrophages: A peroxisome proliferator-activated receptor-γ/δ-mediated effect that links lipid metabolism and immunity. J Mol Endocrinol. 2008;22:1394–402.

28. Kiss-Tóth É, Röszer T. PPAR γ in kidney physiology and pathophysiology. PPAR Res. 2008;183108.

29. National research council. Guidelines for the care and use of laboratory animals.8th ed. Washington DC; National academic press. 2011.