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Comparative study on the influence of L carnitine and/or fenofibrate against streptozotocin induced diabetic nephropathy: role of TGF-β1/Smad Signaling Pathway

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Abstract: Background: The most prevalent form of kidney failure is diabetic nephropathy (DN) and subsequent mortality and morbidity. L carnitine (L.C) and fenofibrate have been formerly showed to be potent in treating DN in rats. **Objective:** The current work compares the effectiveness of L.C and/or fenofibrate in streptozotocin (STZ)-induced DN and the mechanism that leads to it. **Materials and Methods:** Thirty male Sprague Dawley rats were subdivided into five groups; Control group, Diabetic (D) group, D/fenofibrate group, D/ L.C group, D/combination (fenofibrate/L.C) group. Streptozotocin as a single dose was used to cause DN in rats by intraperitoneal injection. **Results:** Serum creatinine (SCr) and blood urea nitrogen (BUN) levels were significantly higher in DN rats, although overall antioxidant capacity was significantly lower (TAC). Histopathological tests of the kidney confirmed these biochemical findings on the same axis. Treatment with L.C and/or fenofibrate, on the other hand, clearly strengthened these functional parameters, TAC, and diabetic histological shifts. The transforming growth factor-1 (TGF- β1)/SMAD pathway was used to investigate the potential mechanism of L.C and/or fenofibrate on diabetic kidney injury defense. The findings revealed that diabetic rats treated with L.C and/or fenofibrate had their TGF- β1/SMAD pathway rebalanced. Surprisingly, taking L.C and fenofibrate at the same time had better safety than any medication alone. **Conclusion:** Experiments revealed that L.C and/or fenofibrate can be a powerful agent for stopping the development of DN through inhibiting TGF-β1/SMAD -mediated renal fibrosis, whilst their combination exerted a superior renoprotective effect.

Keywords: L-carnitine, fenofibrate, diabetic nephropathy, TGF-β1/SMAD.

1. INTRODUCTION

Diabetes mellitus (DM) is indeed the dominant cause of end-stage kidney disease (ESRD) all over the world. Despite strict regulation of glycemic and metabolic disorders, the number of diabetic patients entering ESRD due to DM remains as high as 40% [1, 2]. The molecular pathways underlying the evolution of DN are highly complex involving several mediators. Significant players among these have been identified as oxidative stress, chemo-attractants, fibrotic cytokines, and apoptosis [3-5].

Transforming growth factor-β1 (TGF- β1) is a multifunctional cytokine that is involved in the process of developing glomerulo-sclerosis and interstitial fibrosis [6]. TGF- β1 is believed to play an important part in the development of DN [6-8]. TGF-β1 stimulates the biological activities of two essential downstream mediators, SMAD 2 and SMAD 3, such as extracellular matrix (ECM) production, which is inhibited by SMAD 7, the SMAD inhibitor, after binding to its receptor. The role of the TGF-β1/SMAD 3 signaling pathway in mediating renal fibrosis is well known [9, 10], meaning that blocking TGF-β1/SMAD 3 may be a useful technique for

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preventing DN progression. So far, no suitable active therapy for delaying the development of renal failure has been established. As a result, new therapeutic therapies for DN management are needed [11].

Hydroxy—N-trimethyl ammonium-butyrate or L.C is one of the components that results from lipid metabolism and is necessary to make adenosine triphosphate (ATP) via long-chain fatty acid - oxidation. As a consequence, L.C serves as an antioxidant in an indirect way, facilitating fixing the oxidized membranes or lipid bilayers[12,13]. Furthermore, L.C is considered as a direct scavenger for both H_2O_2 and O_2 [14]. There is overwhelming evidence that L.C supplementation, by its antioxidative action, protects against insulin resistance, diabetic podocyte injury, and diabetes-induced endothelial dysfunction [15,16]. However, the molecular mechanism that underlies the advantages of L.C for DN caused by STZ remains unexplored.

Fibrates are peroxisome proliferator activated receptor alpha (PPAR-alpha) agonists was heavily studied for their tolerability and defense effects on lipid-lowering process, inhibition ability regarding atherosclerosis formation, and ability to prevent DN [17]. The main compounds are bezafibrate, ciprofibrate, clofibrate, fenofibrate, and gemfibrozil[17,18]. Fenofibrate treatment has been said to improve renal structure and function by lowering glomerular hypertrophy and the mesangial matrix associated with type 1 or type 2 diabetes. Fenofibrate prevented TGF-β1activation in cultured mesangial cells treated with elevated glucose levels. The TGF-β1/SMAD pathway's purpose was to promote inflammation, induce renal fibroblast proliferation, and synthesis a variety of ECM proteins, including collagens[19]. Besides that, the mechanism of fenofibrate's beneficial effect in the prevention of DN has not been precisely interpreted.

The preventive role of L.C and/or fenofibrate in the prevention of DN was routinely validated in this research. Furthermore, for the first time, the potential pathway through TGF-β1/SMAD signaling was explored.

2. METHODS

2.1 Animals

A stable environment with a specific temperature (23°C), humidity (60-10%), and a light/dark (12:12 h) loop (National Institute for Science, Cairo, Egypt) was provided to keep the adult male Wistar rats weighing 150-200 g. An unlimited permission to both; water ad libitum and regular rat chow was given for one week prior to the experimental experiments. We gained approval for all of the experimental methods by the animal ethics committee of the Faculty of Pharmacy's (No. 113) in Cairo, Egypt.

2.2 Chemicals

All of the used chemicals were of reagent-grade efficiency, with the exception of STZ, as we brought it from Sigma Chemical Co. (St. Louis, MO, USA). Minapharm (Cairo, Egypt) supplied fenofibrate, and Mepaco supplied the L.C (Cairo, Egypt

2.3 Diabetes induction

Freshly formulated STZ was injected in a single dose (52.5 mg/kg, IP) in a sodium citrate (10mM) buffer, 4.5 pH, was given to animals, after fasting for 12 hours. For the prevention of early mortality, as a result of insulin rash that is released after the destruction of beta cells of the pancreas, rats were given glucose in drinking water (15 g/L) for 24 hours after the STZ injection. After 72 hours, a blood glucose level of more than 250 mg/dl was marked as diabetes [20].

2.4 Design of the work

Six groups (n=6) have been established from the diabetic rats at random: (1) A control group has been formed and rats were provided single I.P injection of citrate buffer (0.1 M, pH 4.5). (2) Diabetic (DN) group. STZ (52.5 mg/kg) was been given to the rats by intraperitoneal injection as a single dose; and (3) DN/ fenofibrate group. Rats with diabetes were given fenofibrate in a dose of 25mg/kg for six weeks; (4) DN/ L.C group; the diabetic rats were given L.C in a dose of 200 mg/kg for six weeks; (5) DN/combination (fenofibrate /L.C). At the completion of the trail, diabetic rats had been fasted overnight, and their final fasting blood glucose levels and body weight had been registered for every animal. Blood samples were taken under moderate anaesthesia by retro-orbital sinus puncture, with isolation of the serum to do the biochemical analysis. Before they were processed, the samples were kept in a -80°C freezer. Kidneys were removed then cleaned by normal saline and dried by a filter paper before measuring its weight. For histological examinations, we fixed the right kidneys in 10% neutral buffered formalin, while the left ones were removed for further study.

2.5. Evaluation of metabolic variables:

2.5.1. Body weight:

The body weight of animals in each group were determined and expressed in grams at the start and at the end of the experiment**.**

2.5.2. Kidney weight index (KWI):

Rats were weighed before sacrifice and the kidney was weighed after sacrifice. The KWI was calculated following this equation:

Organ weight index = (rat organ weight / rat body weight) X 100 21.

2.5.3. Fasting blood glucose level:

Micro-capillaries were used to extract blood from rats after an overnight fasting (12 hours) from the retro-orbital plexus under light ether anesthesia (Optilab, Berlin, Germany). In order to calculate FBG, a drop of blood was placed on the glucometer strip loaded in a single-touch glucometer, (ACCU-Check Active, Roche Diagnostics, Germany).

2.6. Evaluation of biochemical parameters in serum:

2.6.1. Urea & creatinine level

In order to test serum creatinine and urea, we used commercial kits (Biodiagnostic Co., Egypt) by spectrophotometer (UV-1700 Spectrophotometer, Shimadzu, Japan).

2.7. Total antioxidant capacity (TAC):

Using a buffer with hypotonic lysis [0.2 percent Triton-x-100, 10 mM tris-base, 1 mM EDTA (sodium salt)], 10% homogenates have formulated. We then made centrifuging at 4000 rpm for 15 minutes at 4 C by applying a colorimetric kit (biodiagnostic Co., Egypt). Then the supernatant was collected for TAC determination as demonstrated by instruction of the manufacturer [22].

2.8. Examination of histopathology:

Right kidneys were instantly set using a10% neutral buffered formalin after they were excised, dehydrated in ethanol in an ascending matter regarding their grades, cleared in xylene, and set in paraffin. Haematoxylin and eosin were used to dye sections of 3 μm thickness [23].

A professional histopathologist blinded to the identification of the analysed samples conducted both examination of specimens and histopathologic processing.

2.9. Evaluation of TGFβ-1 contents in the kidney using ELISA:

Through applying ELISA kits, renal contents of TGF-β1 were tested and calculated as pg/mL. The manufacturer's guidelines for the used kits were followed to the letter.

2.10. Western blot assay for the estimation of P-SMAD protein expression in the kidney:

RIPA (radioimmunoprecipitation assay) lysis buffer (Tris base 20 mmol/L, NaCl 150 mmol/L, EDTA 1 mmol/L, EGTA 1 Mm, NP40 1 percent, and Na deoxycholate 1 percent) complemented by protease and phosphatase inhibitors was used to homogenise kidney tissue. The Bradford procedure was used to assess protein concentrations. On a 7 percent SDS-PAGE gel, equivalent quantities of cell lysates were fractionated and transferred to nitrocellulose membranes (Bio-Rad). At room temperature the membranes were blocked for 2 hours in 7.5 percent non-fat dry milk in TBST (0.05 percent Tween-20 Tris-buffered saline), then sub cultured with primary antibodies overnight at 4°C. P-SMAD (1:200 dilution) and β-actin (dilution 1:5000) were the main antibodies used. After cleaning, the membranes were incubated for 1 hour at room temperature by using secondary horseradish peroxidaseconjugated antimouse IgG antibody (1:5000, Bio-Rad), then washed again. Enhanced chemiluminescence (ECL Plus; Amersham, Arlington Heights, 13 IL, USA) was used to image proteins, and densitometry and Molecular Analyst Software were used to quantify them (Bio-Rad). The amount of protein was measured in terms of β-actin.

2.11. Statistical analysis

The findings are shown as means ± SE, and the difference among groups was investigated using one-way ANOVA with Tukey-Kramer as a complementary test. Statistical significance was determined at a p value < 0.05.

3. RESULTS

3.1 Effects of fenofibrate and\or L.C on kidney -to – body weight (KW/BW) ratio in STZ induced DN in rats:

As shown in figure 1, -KW/BW ratio has significant elevation by 162.07% within the DN group in comparison with the control group. However, pretreatment with either fenofibrate (25mg/kg/day/P.O) or L.C (200 mg/kg/day/P.O) significantly decreased KW/BW ratio by 30.35% and 31.58% respectively, compared to DN group.

A further marked significant reduction in KW/BW ratio by 49.59 % was produced upon treatment with combination compared to DN group, also a significant decreasing in KW/BW ratio by 27.95% and 26.33% compared to DN animals treated with either fenofibrate or L.C alone, respectively.

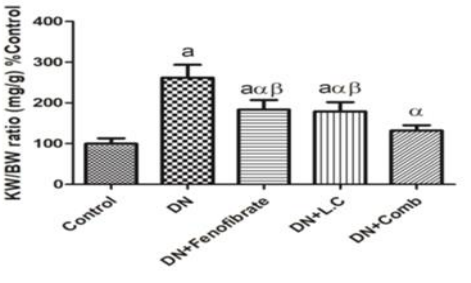

Figure 1. Effects of fenofibrate and\or L.C on kidney -to – body weight (KW/BW) ratio in STZ induced diabetic nephropathy in rats. Data are expressed as mean ± SE. a,α,β is significantly different from the control group, DN group and DN + comb treated group respectively at P < 0.05 using ANOVA followed by Tukey.

3.2. Effects of fenofibrate and\or L.C on blood glucose level (mg\dl) in STZ induced DN in rats:

Glucose level was significantly increased by 388.11% in DN group in comparison with the control group.

Giving the diabetic rats either fenofibrate (25mg/kg/day/P.O) or L.C (200mg/kg/day/P.O) or combined regimen had not decrease the glucose level in comparison with the DN group as shown in in fig (2).

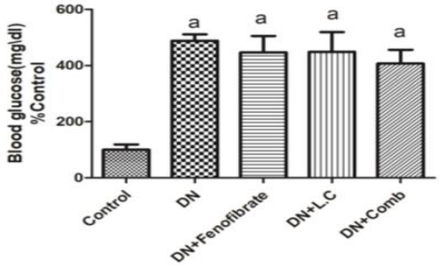

Figure 2. Effects of fenofibrate and\or L.C on blood glucose level (mg\dl) in STZ induced diabetic nephropathy in rats**.** Data are shown as mean ± SE. a,α,β have significant difference compared to the control group, DN group and DN + comb treated group respectively using ANOVA test at a P-Value < 0.05 followed by Tukey.

3.3. Effects of fenofibrate and\or L.C on serum creatinine &urea level in STZ induced DN in rats:

As in comparison to the control group serum creatinine and blood urea nitrogen levels had been considerably elevated ($p<0.05$) by 78.60% and 158.21%, respectively (Table 1). Interestingly, both fenofibrate or L.C remedy theoretically alleviated DN, as validated via way of means of a widespread drop in serum creatinine ranges of 57.36 percentage and 68.35 percentage, respectively, and blood urea nitrogen ranges 42.27 percent and 49.63 percent, respectively, relative to the DN group.

In comparison with the DN group, combined therapy resulted in a more substantial decrease in creatinine and blood urea nitrogen levels of 82.64 percent and 60.78 percent, respectively.

Table1. Effects of fenofibrate and\or L.C on Serum creatinine &urea level in DN induced by STZ in rats:

Treatment Groups	Serum creatinine (mg/dl)	Blood urea nitrogen (mg/dl)
(1) Control	0.158± 0.0145	42.6± 2.63
(2) DN	1.40± 0.0258^{a}	110± 1.84^{a}
(3) DN +fenofibrate	0.597± 0.0285$^{a\alpha\beta}$	63.5± 1.04$^{a\alpha\beta}$
(4) DN +L.C	0.443± 0.0305 $^{a\alpha\beta}$	55.4± 1.63
(5) DN+ Comb	0.243± 0.0202$^{\alpha}$	43.2± 0.932$^{\alpha}$

Data are expressed as mean ± SE. a,α,β is substantially distinct from the control group, DN group and DN + comb treated group respectively at $P < 0.05$, the usage of ANOVA accompanied with Tukey.

3.4. Effects of fenofibrate and\or L.C on kidney TAC level in DN induced by STZ in rats:

Total antioxidant ability degree becomes notably reduced by 64.07% in DN group compared to control group as proven in figure 3.

Pretreatment with either fenofibrate or L.C notably improved kidney overall antioxidant ability stage by 79.29% and 60.61% respectively, as in comparison to DN group, similarly marked substantial growing in overall antioxidant ability level by 138.88% become produced upon remedy with combination, as in comparison to DN group.

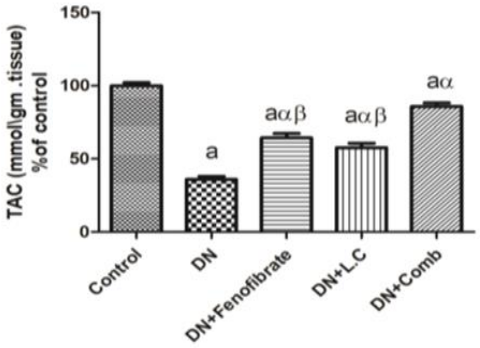

Figure 3. Effects of fenofibrate and\or L.C on TAC in DN induced by STZ in rats. Data are shown as mean ± SE. a,α,β is have an extensive difference between the control group, DN group and DN + comb treated group respectively using ANOVA test at a P-value < 0.05 followed by Tukey.

3.5. Fenofibrate and/ or L.C restored histopathological features in DN induced by STZ in rats:

The control group had normal architecture, while the diabetic group had oedematous epithelial lining with lack of brush boundaries, intra-tubular debris, and hyaline casts in the proximal tubules, as seen in figure 4. Furthermore, in the diabetic group, the renal capsule showed significant disturbance, as well as dispersed atrophied glomeruli. Rats in the DN+ comb community, on the other hand, showed a major improvement in their kidney architecture.

3.6 Effects of fenofibrate or L.C on renal TGF-β1 level in DN induced by STZ in rats

Renal TGF-β1 level in DN group was significantly increased by 130.53% when compared to the control group.

Giving either fenofibrate or L.C significantly decreased renal TGF-β1 level by 40.95% and 36.28% respectively, compared DN group.

A further marked significant reduction in renal TGF-β1 level by 45.34% was produced upon treatment with combination, compared to DN group.

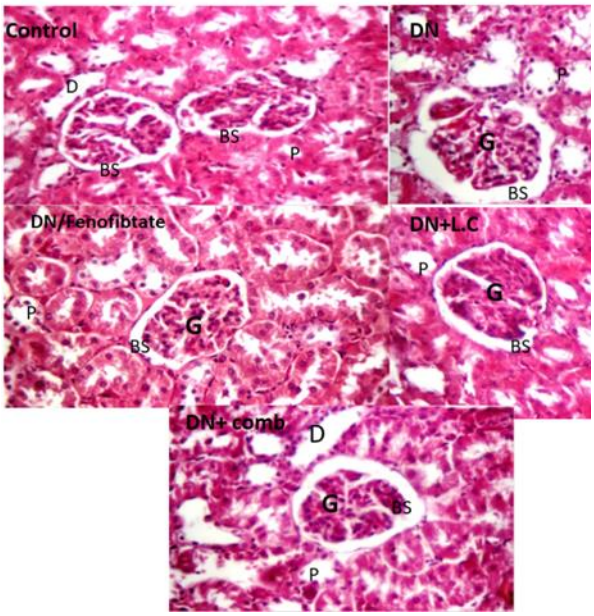

Figure 4. Haematoxylin and eosin staining of renal tissue seen by light microscopy. H&E was used to dye kidney samples. The tissues in the DN group (400x) had oedematous epithelial lining with lack of brush borders with debris intra-tubular, hyaline casts in proximal tubules and marked disturbed renal capsule, dispersed atrophied glomeruli, whereas the control parts (400x) showed no histological alterations. Few small glomeruli with hypercellularity and comparatively expanded Bowman's spaces were seen in the DN+ fenofibrate or DN+L.C (400x) group, and some proximal tubules displayed brush boundary loss. However, there were average renal capsule, and only a few proximal tubules displayed brush boundary loss (long arrow).

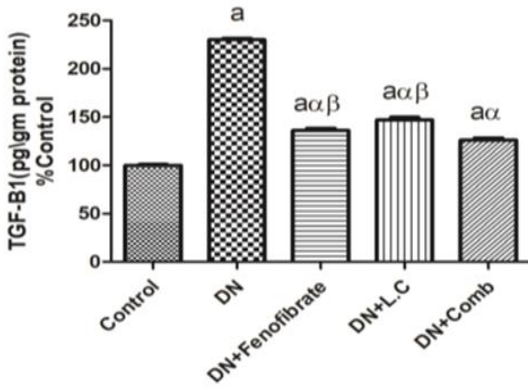

Figure 5. Effects of fenofibrate and\or L.C on renal TGF-β1 level in DN induced by STZ in rats: Data are expressed as mean ± SE. a,α,β is significantly different from the control group, DN group and DN + comb treated group respectively using ANOVA accompanied by Tukey at $P < 0.05$.

3.7. Effects of fenofibrate and\or L.C on expression of renal phosphorylated SMAD (P-SMAD) level in DN induced by STZ in rats:

As shown in figure 6, Renal P-SMAD expression was significantly upregulated by 699% in DN group when compared to control group.

-When diabetic nephropathy rats were pretreated with either fenofibrate or L.C, the expression of P- SMAD was reduced by 34.91 % and 41.63 %, respectively, compared to the DN group, while combination resulted in marked down regulation of P- SMAD expression by 54.94% compared to DN group.

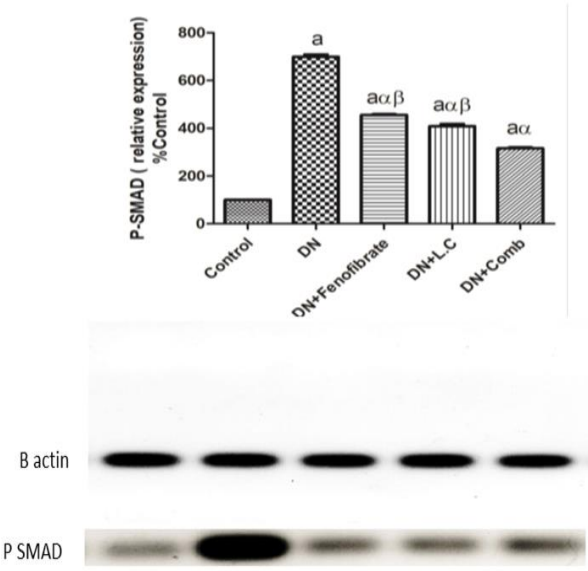

Figure 6. Effects of fenofibrate and\or L.C on expression of renal Phosphorylated SMAD (P-SMAD) level in DN induced by STZ in rats: Data are shown as mean ± SE. a,α,β had significant difference from the control group, DN group and DN + comb treated group respectively using ANOVA accompanied by Tukey at $P < 0.05$.

4. DISCUSSION

Diabetic nephropathy is caused by a complicated and multifactorial mechanism that looks to be aggregate of hemodynamic and metabolic factors, together with extended angiotensin II and hyperglycemia[24]. Hyperglycemia, however, is commonly taken into consideration to be the number one beginning aspect in DN development [25].

It is consequently crucial to discover preventive measures towards DN. Fenofibrate, the agonist of PPAR-alpha and L.C, a cofactor vital for the delivery of long-chain fatty acids into mitochondria for strength manufacturing in peripheral tissues; their impact has been investigated in the present study [26], on diabetic nephropathy in rats. Hyperglycemia caused hemodynamic and metabolic abnormalities,

including increased advanced glycation end products (AGEs) formation and enhanced reactive oxygen species (ROS) generation, during the initiation and progression of DN [27].

We confirmed that pre-remedy with fenofibrate and L.C mitigated the diabetes-triggered kidney injury, as proven through enhancing the parameters of kidney feature and attenuating the deterioration in the morphology of different kidney sections resulting from diabetes. These results are in contrast with a previous study where they had a look at where in fenofibrate appreciably suppresses fibrosis through improving fatty acid oxidation in a mouse version of continual kidney disease [28]. In addition, it turned into formerly proven that L.C remedies have a protective effects regarding potential renal and pancreatic harm resulting from cyclosporine A in rats[29]. This protective effect to the kidney of L.C can be further explained by the prevention of injury resulting from ischemia and following reperfusion[30] as well as glycerol- and contrast-triggered nephropathy [31, 32]. In our study, the induction of STZ nephropathy turned into highlighted through multiplied renal markers, i.e. serum urea nitrogen and creatinine, which have been constant with evidence from STZ [33].

Whilst pretreatment with fenofibrate and/or LC substantially lowered serum creatinine and BUN, renal hypertrophy was improved and renal tissue degradation was reduced, which was consistent with two previous studies [34,35].

Moreover, STZ administration triggered oxidative stress, as demonstrated by reduced overall antioxidant capacity and increased ratio of kidney weight/body weight, a main indicator for body weight reduction and increased renal size [36] relative to control rats, which is related to early researches, reporting that STZ-prompted diabetes in animals displayed a mentioned decline in body weight and significant increase in both kidney weight index and fasting blood glucose level[37,38]. Also, it's been indicated that STZ brought about diabetic rats confirmed a massive upward thrust in kidney to frame weight ratio in comparison to rats in control group [39].

The diabetic kidney is characterized through immoderate development of ROS, generally because of expanded NADPH oxidase pastime and intensified superoxide mitochondrial production, as a result of increased metabolic activity and mitochondrial protein glycation [40].

This significant decline in STZ-induced DN-associated BW can be explained by hyperglycemia, hypoinsulinemia, increased muscle loss and tissue protein loss [36,41]. The initial phase of clinical and experimental DN is marked by hypertrophic changes in all renal compartments, including renal and glomerular size increases. STZ's diabetogenic (hyperglycemic) activity may be due to its β-cell-cytotoxic effects through inhibiting insulin development and selectively killing the beta-cells that generate insulin through causing necrosis [42].

In our results, fenofibrate and/ or L.C pretreatment increased total antioxidant capacity and significantly reduced the altered KW/BW ratio compared to diabetic rats. This statistic revealed that antioxidant associated pathways can be improved by fenofibrate and/ or LC, as a consequence defensive the kidney in opposition to DN. Obviously, fenofibrate seems to relieve oxidative stress by preventing inflammation and lowering NOX in hypertensive rats [43].

Fenofibrate has been announced to play a key function in lowering oxidative stress with inside the kidneys of spontaneously hypertensive rats by decreasing p38 MAPK and c-Jun N-terminal kinase (JNK) phosphorylation signal, lowering renal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and rising Cu-Zn-superoxide dismutase activity [43].

Regarding L.C, free radical yielding has been blocked, assisting to discourage impairment of mitochondrial fatty acid beta-oxidation and shielding tissues from damage with the aid of restoring oxidized membrane lipids [26]. L.C has additionally been identified as an O2- and H2O2 direct scavenger [14]. There is overwhelming proof that L.C supplementation performs a useful position in protecting towards insulin resistance and diabetes-prompted endothelial disorder thru its antioxidant effects [15, 16].

In a wide range of progressive kidney illnesses including DN, fibrosis of the kidney, is a common endpoint that ends in nephron degradation and is diagnosed through macrophages invasion, stimulation of fibroblast and immoderate accumulation of ECM that may contribute to end-stage renal dysfunction's development [44,45].

TGF-β1 is a primary facilitator for the fibrosis of the kidney as it starts the intracellular signals by phosphorylating and nuclear translocating SMAD 3, which is specifically linking the promoting region of the ECM molecules thus encourages the development of renal fibrosis [46]. Moreover, TGF-β1 affects another I-SMAD called SMAD 7 that adversely adjusts the SMAD 3 activation [47].

In preceding study TGF-β1/SMAD pathway has been diagnosed in renal fibrosis [48]. Thus, the current we aimed to observe the renoprotective outcomes of fenofibrate and/ or L.C that target the TGF-β1/SMAD pathway.

Our work revealed that diabetic group confirmed considerable growth in renal TGF-β1 level & P-SMAD protein expression which become notably reduced with the aid of using pre-remedy

with fenofibrate and/ or L.C. These results advocate that fenofibrate and L.C might also additionally have anti fibrotic effects. By blocking off PAI-1 and TGFβ1 within the renal cortex, which in the end results in much less extracellular matrix deposition in addition to TGF-β1/SMAD signaling pathways in diabetes mellitus, a renoprotective effect at both cellular and molecular levels can be achieved by fenofibrate[49]. Other studies have proven that fenofibrate gets rid of diabetic renal harm with the aid of using inhibiting the signaling pathways of TGF-β1/SMAD 3 and NF-kB with next repression of fibrosis and irritation in diabetic nephropathy [19].

Meanwhile, in experimental diabetes, L.C extensively averted renal fibrosis, as visible through a big lower in renal TGFβ1 levels consistent with renoprotective results of L.C suggested in injury from ischemia-reperfusion [30], incurable cyclosporine nephropathy [50], and doxorubicin-induced nephritic syndrome [51]. Moreover, in the removed kidneys of the rat, we observed both; the anti-inflammatory and anti-fibrotic results of L.C [52].

5. CONCLUSIONS

In conclusion, the current research hypothesized that a combination of L.C and fenofibrate provided the best defense against STZ-induced DN. Also it confirmed that the down regulation of TGF-β1/SMAD pathway performs a vital protection in mediating the renodefensive impact of fenofibrate and L.C in diabetic rats followed by anti-oxidant effects. Since, consistent with in advance research, fenofibrate and L.C have exerted profound results in continual kidney disease [34, 35].

In order to explore the therapeutic action of fenofibrate and L.C on inflammatory pathways in diabetic rats, further research is required.

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Conflicts of Interest: The authors have declared that there is no conflict of interest.

Ethical Statement: The Ethics Committee at the Faculty of Pharmacy, Al-Azhar University (permit number: (No. 113/2017) approved all procedures used in this research. The inquiry followed the US National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011).

Author Contribution: The specific contributions made by each author: Amany A. Alzokaky, Nahed A. Raslan and Nayira A. Abdel Baky conceived and designed the study. Marwa A. Ahmed, Amany A.Alzokaky, Nahed A. Raslan and Nayira A. Abdel Baky acquired the data. Marwa A. Ahmed, Amany A. Alzokaky, Nahed A. Raslan and Nayira A. Abdel Baky analysed and interpreted the data. Amany A. Alzokaky and Nahed A. Raslan drafted the manuscript. Nayira A. Abdel Baky critically revised the manuscript.

List of Abbreviations: BUN; Blood urea nitrogen, DN; Diabetic nephropathy, ECM; Extracellular matrix, D; Diabetes mellitus, ESRD; End-stage renal disease, FF; Fenofibrate, KWI; Kidney weight index, KW/BW; Kidney weight /body weight ratio, L.C; L-carnitine. PPAR-α; Peroxisome proliferator-activated receptor alpha, P-SMAD; Phosphorylated Smad, AGEs; advanced glycation end-product, ROS; Reactive oxygen species, STZ; Streptozotocin, TAC; Total antioxidant capacity, TGF-β; Transforming growth factor beta, ATP; Adenosine triphosphate, NF-kB; Nuclear factor- kappa B.

REFERENCES

1. Ahn JH, Yu JH, Ko S-H, Kwon H-S, Kim DJ, Kim JH, et al. Prevalence and determinants of diabetic nephropathy in Korea: Korea national health and nutrition examination survey. Diabetes Metab J. 2014;38(2):109–19.

2. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care. 2014;37(10):2864–83.

3. Jin H, Piao SG, Jin JZ, Jin YS, Cui ZH, Jin HF, et al. Synergistic efenofibrateects of leflunomide and benazepril in streptozotocin-induced diabetic nephropathy. Nephron Exp Nephrol. 2014;126(3):148–56.

4. Qi XM, Wu GZ, Wu YG, Lin H, Shen JJ, Lin SY. Renoprotective efenofibrateect of breviscapine through suppression of renal macrophage recruitment in streptozotocin-induced diabetic rats. Nephron Exp Nephrol. 2006;104(4):e147-57.

5. Stephen TL, Rutkowski MR, Allegrezza MJ, Perales-Puchalt A, Tesone AJ, Svoronos N, et al. Transforming growth factor β-mediated suppression of antitumor T cells requires FoxP1 transcription factor expression. Immunity. 2014;41(3):427–39.

6. Wang W, Huang XR, Li AG, Liu F, Li J-H, Truong LD, et al. Signaling mechanism of TGF-beta1 in prevention of renal inflammation: role of SMAD 7. J Am Soc Nephrol. 2005;16(5):1371–83.

7. Boak AM, Roy R, Berk J, Taylor L, Polgar P, Goldstein RH, et al. Regulation of lysyl oxidase expression in lung fibroblasts by transforming growth factor-beta 1 and prostaglandin E2. Am J Respir Cell Mol Biol. 1994;11(6):751–5.

8. Roberts AB, Heine UI, Flanders KC, Sporn MB. Transforming growth factor-beta: Major role in regulation of extracellular matrix. *Ann N Y Acad Sci*. 1990; 580(1 Structure, Mo):225–32.

9. Belghith M, Bluestone JA, Barriot S, Mégret J, Bach J-F, Chatenoud L. TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. Nat Med. 2003;9(9):1202–8.

10. Huang C, Kim Y, Caramori MLA, Fish AJ, Rich SS, Miller ME, et al. Cellular basis of diabetic nephropathy: II. The transforming growth factor-beta system and diabetic nephropathy lesions in type 1 diabetes. Diabetes. 2002;51(12):3577–81.

11. Oujo B, Muñoz-Félix JM, Arévalo M, Núñez-Gómez E, Pérez-Roque L, Pericacho M, et al. L-Endoglin overexpression increases renal fibrosis after unilateral ureteral obstruction. PLoS One. 2014;9(10):e110365.

12. Mate A, Miguel-Carrasco JL, Vázquez CM. The therapeutic prospects of using L-carnitine to manage hypertension-related organ damage. Drug Discov Today. 2010;15(11–12):484–92.

13. Arduini A. Carnitine and its acyl esters as secondary antioxidants? Am Heart J. 1992; 123(6):1726–7.

14. Gülçin I. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006;78(8):803–11.

15. Fan JP, Kim D, Kawachi H, Ha T-S, Han GD. Ameliorating efenofibrateects of L-carnitine on diabetic podocyte injury. J Med Food. 2010;13(6):1324–30.

16. Ringseis R, Keller J, Eder K. Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency. Eur J Nutr. 2012;51(1):1–18.

17. Fazio S, Linton MF. High-density lipoprotein therapeutics and cardiovascular prevention. J Clin Lipidol. 2010;4(5):411–9.

18. Guan Y, Breyer MD. Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease. Kidney Int. 2001;60(1):14–30.

19. Li L, Emmett N, Mann D, Zhao X. Fenofibrate attenuates tubulointerstitial fibrosis and inflammation through suppression of nuclear factor-κB and transforming growth factor-β1/SMAD 3 in diabetic nephropathy. Exp Biol Med (Maywood). 2010;235(3):383–91.

20. Ghasemi A, Khalifi S, Jedi S. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiol Hung. 2014;101(4):408–20.

21. Yang Q, Xie R-J, Luo X-H, Han B, Yang T, Fang L, et al. Expression of PKC in rat hepatic fibrosis and the efenofibrateect of Dan-shao-hua-xian Capsule on its expression pattern. Zhonghua Gan Zang Bing Za Zhi. 2005;13(9):707–8.

22. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54(5):356–61.

23. Banchroft JD, Stevens A , Turner DR. Theory and practice of histological tecniques. 4th ed. Churchill Livingstone, Edinbergh ,London , New York and Tokyo.1996; 273-292 .

24. Reddy MA, Tak Park J, Natarajan R. Epigenetic modifications in the pathogenesis of diabetic nephropathy. Semin Nephrol. 2013;33(4):341–53.

25. Chang C-C, Chang C-Y, Wu Y-T, Huang J-P, Yen T-H, Hung L-M. Resveratrol retards progression of diabetic nephropathy through modulations of oxidative stress, proinflammatory

cytokines, and AMP-activated protein kinase. J Biomed Sci. 2011;18(1):47.

26. Giudetti AM, Stanca E, Siculella L, Gnoni GV, Damiano F. Nutritional and hormonal regulation of citrate and carnitine/acylcarnitine transporters: Two mitochondrial carriers involved in fatty acid metabolism. Int J Mol Sci. 2016;17(6):817.

27. Zelmanovitz T, Gerchman F, Balthazar AP, Thomazelli FC, Matos JD, Canani LH. Diabetic nephropathy. Diabetol Metab Syndr. 2009;1(1):10.

28. Kang HM, Ahn SH, Choi P, Ko Y-A, Han SH, Chinga F, et al. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. Nat Med. 2015;21(1):37–46.

29. Xiang Y, Piao SG, Zou HB, Jin J, Fang MR, Lei DM, et al. L-carnitine protects against cyclosporine-induced pancreatic and renal injury in rats. Transplant Proc. 2013;45(8):3127–34.

30. Liu Y, Yan S, Ji C, Dai W, Hu W, Zhang W, et al. Metabolomic changes and protective efenofibrateect of (L)-carnitine in rat kidney ischemia/reperfusion injury. Kidney Blood Press Res. 2012; 35(5):373–81.

31. Kunak CS, Ugan RA, Cadirci E, Karakus E, Polat B, Un H, et al. Nephroprotective potential of carnitine against glycerol and contrast-induced kidney injury in rats through modulation of oxidative stress, proinflammatory cytokines, and apoptosis. Br J Radiol. 2016;89(1058):20140724.

32. Boyacioglu M, Turgut H, Akgullu C, Eryilmaz U, Kum C, Onbasili OA. The efenofibrateect of L-carnitine on oxidative stress responses of experimental contrast-induced nephropathy in rats. J Vet Med Sci. 2014;76(1):1–8.

33. Alderson NL, Chachich ME, Frizzell N, Canning P, Metz TO, Januszewski AS, et al. Efenofibrateect of antioxidants and ACE inhibition on chemical modification of proteins and progression of nephropathy in the streptozotocin diabetic rat. Diabetologia. 2004;47(8):1385–95.

34. Arora MK, Singh UK.Combination of PPAR-α Agonist and DPP-4 Inhibitor: A Novel Therapeutic Approach in the Management of Diabetic Nephropathy. J Diabetes Metab .2013; 4(10): 320.

35. Abu Ahmad N, Armaly Z, Berman S, Jabour A, Aga-Mizrachi S, Mosenego-Ornan E, et al. l-Carnitine improves cognitive and renal functions in a rat model of chronic kidney disease. Physiol Behav. 2016;164(Pt A):182–8.

36. Zafar M, Naqvi SN. Efenofibrateects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int J Morphol. 2010; 28(1):135–142.

37. Mestry SN, Dhodi JB, Kumbhar SB, Juvekar AR. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. J Tradit Complement Med. 2017;7(3):273–80.

38. Yang M, Zhao L, Gao P, Zhu X, Han Y, Chen X, et al. DsbA-L ameliorates high glucose induced tubular damage through maintaining MAM integrity. EBioMedicine. 2019;43:607–19.

39. Mahajan MS, Upasani CD, Upaganlawar AB, Gulecha VS. Renoprotective Efenofibrateect of Co-Enzyme Q10 and N-Acetylcysteine on Streptozotocin-Induced Diabetic Nephropathy in Rats. Int J Diabetes Clin Res .2020; 7(2) :123.

40. Heyman SN, Rosenberger C, Rosen S, Khamaisi M. Why is diabetes mellitus a risk factor for contrast-induced nephropathy? Biomed Res Int. 2013;2013:123589.

41. Cheng D, Liang B, Li Y. Antihyperglycemic efenofibrateect of Ginkgo biloba extract in streptozotocin-induced diabetes in rats. Biomed Res Int. 2013;2013:162724.

42. Zhang Y, Zhang Y, Bone RN, Cui W, Peng J-B, Siegal GP, et al. Regeneration of pancreatic non-β endocrine cells in adult mice following a single diabetes-inducing dose of streptozotocin. PLoS One. 2012;7(5):e36675.

43. Hou X, Shen YH, Li C, Wang F, Zhang C, Bu P, et al. PPARalpha agonist fenofibrate protects the kidney from hypertensive injury in spontaneously hypertensive rats via inhibition of oxidative stress and MAPK activity. Biochem Biophys Res Commun. 2010;394(3):653–9.

44. Humphreys BD. Mechanisms of Renal Fibrosis. Annu Rev Physiol. 2018;80(1):309–26.

45. Chen L, Yang T, Lu D-W, Zhao H, Feng Y-L, Chen H, et al. Central role of dysregulation of TGF-β/SMAD in CKD progression and potential targets of its treatment. Biomed Pharmacother. 2018; 101:670–81.

46. Huynh P, Chai Z. Transforming growth factor β (TGFβ) and related molecules in chronic kidney disease (CKD). Clin Sci (Lond). 2019;133(2):287–313.

47. Chen HY, Huang XR, Wang W, Li JH, Heuchel RL, Chung ACK, et al. The protective role of SMAD 7 in diabetic kidney disease: mechanism and therapeutic potential. Diabetes. 2011;60(2):590–601.

48. Yang F, Chung ACK, Huang XR, Lan HY. Angiotensin II induces connective tissue growth factor and collagen I expression via transforming growth factor-beta-dependent and -independent SMAD pathways: the role of SMAD 3. Hypertension. 2009;54(4):877–84.

49. Kouroumichakis I, Papanas N, Zarogoulidis P, Liakopoulos V, Maltezos E, Mikhailidis DP. Fibrates: therapeutic potential for diabetic nephropathy? Eur J Intern Med. 2012;23(4):309–16.

50. Bertelli A, Giovannini L, Palla R, Migliori M, Panichi V, Andreini B. Protective efenofibrateect of L-propionylcarnitine on cyclosporine-induced nephrotoxicity. Drugs Exp Clin Res. 1995;21(6):221–8.

51. Boonsanit D, Kanchanapangka S, Buranakarl C. L-carnitine ameliorates doxorubicin-induced nephrotic syndrome in rats. Nephrology (Carlton). 2006;11(4):313–20.

52. Giovannini L, Palla R, Bertelli AA, Migliori M, Panichi V, Andreini B, et al. Cyclosporine nephrotoxicity evaluated by tissue calcium deposition and tubular enzymes is prevented by L-propionylcarnitine in isolated perfused rat kidney. Transplant Proc. 1996;28(6):3122–5.