ORIGINAL ARTICLE

# Lauric Acid Improves Kidney Structure and Function of Streptozotocin-induced Diabetic Nephropathy Rats

Muhammad Aiman Faris Maznan[1], Nursarah Syamimi Anuar[1], Syahirah Ain Shafie[1], Nabilah Ahmad A'ulwi[1], Mohd Daud Bakar[2], Hafandi Ahmad[3], Nurdiana Samsulrizal[1]

[1] TuAH Industrial Research Lab with Bio Fluid Sdn Bhd, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2] Bio Fluid Sdn Bhd, 70, Jalan Bulan U5/172, Subang 2, 40150 Shah Alam, Selangor, Malaysia
[3] Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

## ABSTRACT

**Introduction:** Lauric acid has the potential to reduce blood glucose, stimulate insulin secretion, and enhance antioxidant activity. However, whether lauric acid protects against diabetic nephropathy remains elusive. The study aimed to investigate the nephroprotective effects of lauric acid in streptozotocin-induced diabetic nephropathy rats. **Materials and methods:** Lauric acid was orally administered to diabetic rats for 8 weeks at doses of 25, 50, and 100 mg/kg body weight (bwt). Changes in fasting blood glucose (FBG), glucose tolerance, insulin levels, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were investigated. Biochemical and histological examinations were performed to determine treatment effects on the kidneys structure and function. Fourier transform infrared (FTIR) spectroscopy combined with chemometric analysis was employed to identify potential infrared spectral biomarkers. **Results:** We showed that all treatment doses increased SOD and CAT levels as well as decreased serum creatinine levels and blood urea nitrogen (BUN). However, a significant decrease in FBG levels and an increase in insulin levels were observed exclusively in the DLA50 and DLA100 groups. Meanwhile, only DLA50 animals exhibited normalized MDA levels, increased glomeruli size, and had well-defined tubules. These results are corroborated by findings obtained from hierarchical cluster analysis (HCA) and principal component analysis (PCA) for FTIR peak intensity at wavenumbers 1511 and 1545 $cm^{-1}$. **Conclusion:** Our findings suggest that lauric acid exerts nephroprotective effects in diabetic rats by improving antioxidant activities and positively influencing glucose and insulin levels. These insights contribute to the therapeutic potential of lauric acid in mitigating diabetic nephropathy.
*Malaysian Journal of Medicine and Health Sciences* (2025) 21(3): 337-345. doi:10.47836/mjmhs.21.3.39

**Keywords:** Creatinine, FTIR, Insulin, Lauric acid, Oxidative stress

**Corresponding Author:**
Nurdiana Samsulrizal, Ph.D
Email: nurdiana7251@uitm.edu.my
Tel: +603-5544 4526

## INTRODUCTION

Diabetic nephropathy (DN) refers to the progressive decline in kidney function. It is a common complication of type 1 and type 2 diabetes as a result of hyperglycaemia-induced oxidative damage [1]. Treatment typically involves controlling blood glucose, blood pressure, and cholesterol levels [2]. Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and diuretics are the available treatment options to reduce the risk of further kidney damage [3]. Despite efforts to mitigate, approximately 50% of the patients with type 1 diabetes and 30% of those with type 2 diabetes develop nephropathy and subsequently require dialysis or kidney transplantation [4]. This scenario setting suggests that discovering a new nephroprotective agent for diabetics deserves serious consideration.

Oxidative stress plays a key role in the development and progression of DN [5]. An imbalance between free radical production and endogenous antioxidants triggers the activation of various signalling pathways, which in turn result in structural and functional abnormalities in the kidneys [6]. Malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) are the key markers of oxidative stress in DN [7]. Increased levels of MDA indicate lipid peroxidation, while decreased activities of SOD and CAT reflect impaired antioxidant defence mechanisms [8]. The potential of antioxidants to improve kidney structure and function has been widely demonstrated in animal models and patients with DN [9,10]. Fatty acids are recently receiving special attention for their potential as natural antioxidants to

prevent cellular oxidative damage. Medium-chain fatty acids (MCFAs) were reported to exhibit better antioxidant capacity than long-chain fatty acids (LCFAs) [11]. It has been demonstrated that MCFA can enhance antioxidant capacity [12], prevent systemic inflammation [13], and regulate glucose and lipid metabolism [14].

Lauric acid is a saturated MCFA that is highly present in coconut oil, accounting for approximately 40–50% [15]. It has numerous health benefits, including antidiabetic, antioxidant [16], and anti-inflammatory properties [17]. Our previous study has shown that treatment with lauric acid in the dose range of 25–100 mg/kg bwt reduces oxidative stress-induced male reproductive dysfunctions in STZ-induced diabetic rats [18]. The potential of lauric acid to scavenge free radicals and protect cells against oxidative stress has also been reported in animal and human cells [19,20]. Despite its health-promoting properties, there is no scientific evidence to suggest the potential of lauric acid in preventing or managing DN.

Fourier transform infrared (FTIR) spectroscopy offers tremendous potential for improving diagnosis. Previous studies have demonstrated that FTIR spectroscopy coupled with chemometrics is able to identify the specific molecular fingerprint of biological samples, which in turn provides clues to biochemical and pathological changes [21,22]. FTIR spectroscopy together with chemometric analyses such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) was also reported to be capable of predicting proteins involved in the progression of diabetes [23]. Therefore, this study was designed to examine the feasibility of FTIR analysis combined with chemometric analysis to assess the nephroprotective effects of lauric acid in diabetic rats.

## MATERIALS AND METHODS

### Chemicals

Enzyme-linked immunosorbent assay (ELISA) kits (insulin, malondialdehyde, superoxide dismutase, catalase, and creatinine), eosin, ethanol, hematoxylin, ketamine, lauric acid (R&M Chemicals, Malaysia), paraffin wax, phosphate buffer solution (PBS), saline (1% sodium chloride), sodium citrate buffer (pH 4.5), streptozotocin (STZ) (Sigma-Aldrich, Deisenhofen, Germany), sucrose solution, tween 20 (Sigma-Aldrich, St. Louis, MO, USA), xylazine, and xylene.

### Animals

A total of 30 male Sprague Dawley rats weighing around 250–300 grams at the age of 7 weeks were used in the study. Rats are purchased from Chenur Supplier Sdn. Bhd. Serdang, Selangor. Three animals were placed inside a polypropylene cage (48 x 28 x 36 cm) lined with wood shavings (CHIPSI Classic, Rosenberg, Germany). Animals were kept in a controlled environment of a 12: 12-hour light:dark cycle with a temperature of 25 ± 3 °C upon arrival. The rats were allowed to acclimatize for seven days to become acquainted with their new surroundings. Acclimatization is vital to ensure reproducible experimental results as well as provide better welfare for the rats. The rats were fed with standard rat pellets from Gold Coin Feedmills Sdn. Bhd. The pellets are made from a mixture of corn, vegetables, soybean meal, oil, animal protein, salt, calcium carbonate, antimicrobials, and other grains and grain by-products. The pellets were replaced daily to prevent mold and maintain freshness and the drinking bottles were cleaned every three days to prevent the development of microbes. The rats also had access to enrichment in the form of artificial tunnels for leisure activities. All study protocols, including diabetes induction and sacrifice operation, were approved by the Committee on Animals for Research and Ethics, Universiti Teknologi MARA, Shah Alam (UiTM CARE: 410/2023) and performed in strict accordance with the institutional guidelines and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0.

### Induction of hyperglycaemia

Following the acclimatization period, 0.5 mL of STZ (Sigma-Aldrich, Deisenhofen, Germany) was injected intravenously into each rat (n = 24) at a dosage of 40 mg/kg bwt [18]. Animals were fasted overnight and given only water to drink before injection. A fasting protocol aids in minimizing the competition between STZ and glucose at GLUT2 transporters, thus increasing the success rate of the animal model induction [24]. STZ was prepared freshly in a sodium citrate buffer before each injection, and the normal control group received an equivalent volume of the buffer. After the injection, the rats were given standard rat pellets and 10% sucrose water for two days to prevent hypoglycaemic shock. The rats were then fed with standard rat pellets and regular water from days 3–10 after the induction period. Cage bedding of polyuric animals was replaced every day to maintain a clean environment. The blood glucose level was measured using a portable glucometer (Accu-Check® Performa, Roche, Germany). Animals with blood glucose levels above 11.0 mmol/L are considered diabetic and selected for further testing [25], while those below this range were excluded from the study.

### Experimental design

Animals (N = 30) were assigned into five experimental groups of six rats each. The normal control (NC) and diabetic control (DC) rats received saline (0.9% sodium chloride), while lauric acid was administered to the treatment groups (DLA25, DLA50, and DLA100) at doses of 25, 50, and 100 mg/kg bwt, respectively [18]. The dose range was determined based on our preliminary in vitro study. We found that lauric acid has a half-maximal inhibitory concentration ($IC_{50}$) similar to acarbose. As the approved oral dosage of acarbose by the U.S. Food and Drug Administration (FDA) is 25, 50, and 100 mg, these ranges were selected for this

study [26]. Lauric acid was first dissolved in Tween 20 (Sigma Aldrich, St. Louis, MO, USA) at a ratio of 1:2 to enhance its solubility. A different concentration was then prepared by diluting the mixture in saline before treatment [27]. All treatments, including saline, were given via oral gavage at a volume of 1 mL per 100 g of bwt for 56 days.

**Oral glucose tolerance test**

OGTT was conducted on day 56 following the method provided by [28]. The animals fasted overnight before the test to obtain the baseline glucose level (0 minutes). Each animal was force-fed with 2 g of glucose per kg of body weight dissolved in water. Blood sugar levels were measured at 0, 30, 60, 90, and 120 minutes after glucose administration by using Accu-Check® Performa blood glucose monitors.

**Blood and organ collection**

On day 58, the animals were fasted overnight to ensure their blood sugar level was consistent [29]. The rats were injected intraperitoneally with ketamine and xylazine (0.01 mL/g bwt) for anaesthesia before terminal exsanguinations. A cardiac puncture was performed on each rat to collect 10 mL of blood [30]. The blood samples were centrifuged at 4000 rpm for 15 minutes to obtain the serum, and aliquots were stored at -80 ºC for later analysis. The kidneys were harvested subsequently for biochemical, histological, and FTIR analyses. The left kidney of each animal was stored at -80 °C for biochemical and FTIR analyses, while the right kidney was fixed in 10% neutral buffered formalin before proceeding to the histology study.

**Biochemical evaluations**

Insulin, MDA, SOD, and CAT levels in the serum and kidney were determined by the ELISA Kit 96T (Qayee Bio-Technology Co., Ltd). These evaluations were conducted by following the protocols provided by the manufacturer. Absorbance reading was measured at 540 nm using an Epoch 2 microplate reader (BioTek Instruments, Inc., Vermont, USA). Serum creatinine and BUN levels were determined using a Chemistry Anlayser Automatic (HITACHI 902®) at the Laboratory of Veterinary Clinical Hematology & Biochemistry Department of Pathology & Microbiology, Universiti Putra Malaysia.

**Histology assessment**

The kidney tissues were embedded into paraffin wax using the HistoCore Arcadia H embedding station (Leica Biosystem, Germany). A fully automated rotary microtome (HistoCore AUTOCUT; Leica Biosystem, Germany) was then used to cut the sections at 5 µm. The HI1220 flattening table (Leica Biosystem, Germany) was used in the mounting process of tissue sections onto the glass slides. The tissue sections were deparaffinized with xylene and slowly rehydrated with ethanol that was diluted by 100%, 95%, 80%, and 70%. Hematoxylin and Eosin (H&E) are used to stain the samples. The slides were examined using a Leica DM3000 LED microscope (Leica Microsystem, Wetzlar, Germany) under a magnification of 40X.

**FTIR and chemometric analyses**

An IR spectroscopy experiment was conducted using a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, USA) that had a focal plane array detector. About 10 µL of the supernatant from the homogenized left kidney in phosphate-buffered saline was placed and spread out in the liquid cell by a micropipette [29]. All FTIR spectra were collected within the range of 4000-600 cm-1. Second derivative spectra were used to prevent the overlapping of absorption components through the Savitzky-Golay algorithm with 32 co-added scans and a spectral resolution of 8 cm-1. Chemometric analysis was conducted to assess the differentiation of kidney IR spectra, which are the HCA and PCA. OriginPro software (OriginLab, Northampton, MA, USA) was used for the observation of spectral derivation, and the observed peaks were fitted and processed. General clustering overviews are obtained through HCA and PCA.

**Statistical analysis**

The results are presented as mean ± SD. One-way ANOVA was performed to compare the differences between groups for all variables. Duncan's multiple comparison tests were used to determine any significant differences between the means at a 95% confidence level. The statistical analysis software used was GraphPad Prism 8, Minitab, and OriginPro.

## RESULTS

**Fasting blood glucose**

Fig. 1a shows the changes in FBG levels of the experimental animals at Weeks 0, 4 and 8. The FBG concentrations of the NC animals remained stable throughout the experimental period. However, the DC group showed a significant increase in final FBG values by 65.76% compared to the initial value at Week 0. A similar trend was observed in the DLA25 group, with a 7.1% increase compared to the initial value. Interestingly, the FBG levels at Week 8 decreased by 24.26% in the DLA50 group and by 18.69% in the DLA100 group compared to pre-treatment levels.

**Glucose tolerance**

The DC group displayed significant glucose intolerance (Fig. 1b), as demonstrated by an increase in the area under the curve (Fig. 1c). Lower AUC values as compared to the DC group were observed in all lauric acid-treated diabetic rats, particularly in the DLA50 group. Treatment with 50 mg/kg bwt of lauric acid decreased the AUC by 49.10%, whereas the DLA25 and DLA100 groups yielded a mean AUC of 2949.70± 136.60 and 2780.30± 119.40, respectively.

| Groups | Fasting blood glucose (mmol/L) | | |
|---|---|---|---|
| | Week 0 | Week 4 | Week 8 |
| NC | $4.77 \pm 0.31^{b,x}$ | $4.10 \pm 0.17^{d,x}$ | $4.24 \pm 0.24^{d,x}$ |
| DC | $19.77 \pm 0.45^{a,y}$ | $32.23 \pm 1.33^{a,x}$ | $32.77 \pm 0.64^{a,x}$ |
| DLA25 | $21.97 \pm 01.95^{a,z}$ | $28.53 \pm 1.17^{b,x}$ | $23.53 \pm 1.83^{b,xyz}$ |
| DLA50 | $20.73 \pm 1.27^{a,xy}$ | $19.40 \pm 1.11^{c,xy}$ | $15.70 \pm 0.90^{c,z}$ |
| DLA100 | $24.93 \pm 1.69^{a,xy}$ | $26.43 \pm 1.14^{b,xy}$ | $20.27 \pm 0.40^{bc,y}$ |

(a)

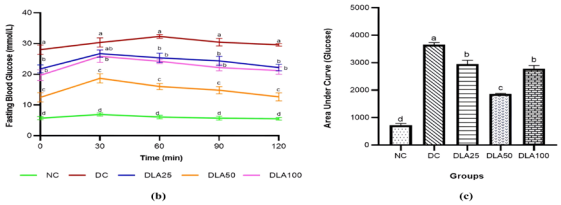


**Fig. 1: Effect of lauric acid on (a) fasting blood glucose level (b) oral glucose tolerance test (OGTT) and (c) area-under-the-curve (AUC) for OGTT. Data are present as mean ± SD for six rats in each group in normal and diabetic group rats. Values with different superscripts a,b,c,d differed significantly at p<0.05 due to treatment effects, whereas values with different superscripts x,y,z differed significantly at p<0.05 due to time effects. NC: normal control; DC: diabetic control; DLA25: diabetic treated with 25 mg/kg bwt of lauric acid; DLA50: diabetic treated with 50 mg/kg bwt of lauric acid and DLA100: diabetic treated with 100 mg/kg bwt of lauric acid.**

## Insulin secretion

The DC group exhibited the lowest serum (Fig. 2a) and kidney (Fig. 2b) insulin concentrations. It is interesting to note that insulin concentrations increased significantly in all lauric acid-treated groups. Treatment with 50 mg/kg bwt of lauric acid results in a 2.42-fold (3.32±0.39 vs 1.37±0.13) and 3.02-fold (5.94±0.12 vs 1.97±0.15) increase in serum and kidney insulin concentration, respectively, as compared to the DC group.

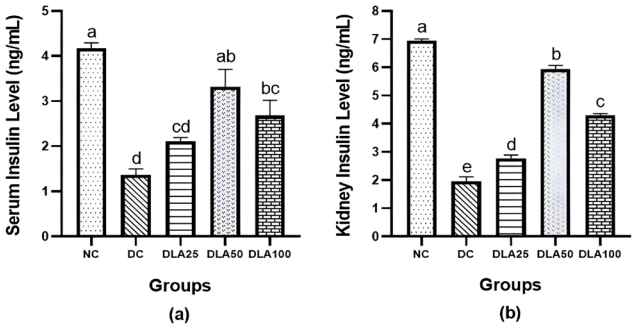


**Fig. 2: Insulin level in (a) serum and (b) kidney. Values are present as mean ± SD for six rats in each group. Values with different superscripts [a,b,c,d,e] differed significantly at p<0.05 due to treatment effects. NC: normal control; DC: diabetic control; DLA25: diabetic treated with 25 mg/kg bwt of lauric acid; DLA50: diabetic treated with 50 mg/kg bwt of lauric acid and DLA100: diabetic treated with 100 mg/kg bwt of lauric acid.**

## Relative kidney weight

As shown in Fig. 3(a), the DC group had the heaviest kidney weight compared to the NC group (1.31 ± 0.10 vs. 0.57 ± 0.05). In contrast, treatment with 25, 50, and 100 mg/kg bwt of lauric acid significantly reduced the relative kidney weight of diabetic rats by 14.67%, 27.72%, and 19.02%, respectively.

## Kidney function markers

To evaluate kidney function, serum creatinine and BUN levels were measured. Fig. 3(b) and 3(c) showed that the DC animals had a significant increase in serum creatinine and BUN levels. However. lower serum creatinine and BUN levels were found in the DLA25, DLA50, and DLA100 groups. The DLA50 rats displayed the lowest serum creatinine (0.97±0.06 vs. 2.13±0.16) and BUN (22.45± 0.38 vs. 47.91± 1.11) compared to the DC group.

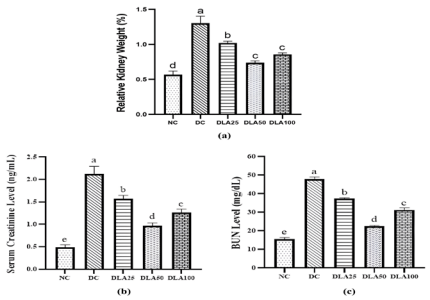


**Fig. 3: Effect of lauric acid on (a) relative kidney weight, (a) serum creatinine and (b) blood urea nitrogen (BUN). Values are present as mean ± SD for six rats in each group. Values with different superscripts a,b,c,d,e differed significantly at p<0.05. NC: normal control; DC: diabetic control; DLA25: diabetic treated with 25 mg/kg bwt of lauric acid; DLA50: diabetic treated with 50 mg/kg bwt of lauric acid and DLA100: diabetic treated with 100 mg/kg bwt of lauric acid.**

## Lipid peroxidation and Antioxidant enzymes

Fig. 4 illustrates that the DC group had the highest concentration of malondialdehyde (MDA) but the lowest SOD and CAT in the serum and kidneys. However, all lauric acid-treated groups displayed a significant decrease in MDA levels and an increase in SOD and CAT levels. A more intriguing finding is that treatment with 50 mg/kg bwt of lauric acid lowered MDA levels to near-normal levels in the serum (0.88±0.08 vs. 1.20±0.08) and kidney (0.20±0.01 vs. 0.25±0.03) of STZ-induced diabetic rats. The SOD increased 2.51-fold (14.50±0.77 vs. 5.77±0.32) and 3.73-fold (8.27±0.13 vs. 2.22±0.16) in the serum and kidney of the DLA50 animals, respectively, as compared to the DC animals. Meanwhile, CAT increased by 78.45% in serum and 72.92% in kidney.

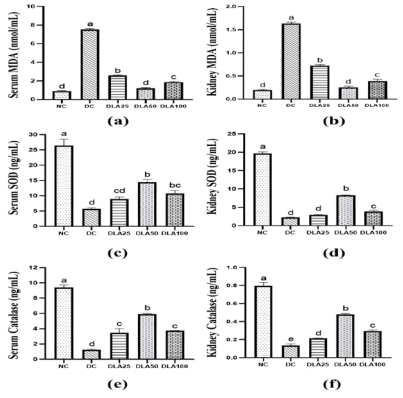


**Fig. 4: MDA, SOD, and Catalase levels in the serum and kidney of rats. Data are present as mean ± SD for six rats in each group. Values with different superscripts [a,b,c,d] differed significantly at p<0.05 due to treatment effects. NC: normal control; DC: diabetic control; DLA25: diabetic treated with 25 mg/kg bwt of lauric acid; DLA50: diabetic treated with 50 mg/kg bwt of lauric acid and DLA100: diabetic treated with 100 mg/kg bwt of lauric acid.**

## Histological examination

A light microscopic examination of the renal cortex revealed the normal cellular structure of the kidney in the NC rats (Fig. 5a). However, the kidney tubules of DC rats (Fig. 5b) are poorly defined, consistent with wider Bowman's spaces (BS) as well as glomeruli shrinkage. A smaller BS and lower kidney tubule necrosis were also observed in all lauric acid-treated groups. Although the DLA50 group displayed normal-sized glomeruli, their kidney tubule remained poorly defined (Fig. 5d). Meanwhile, the DLA100 rats were associated with glomeruli enlargement (Fig. 5e).

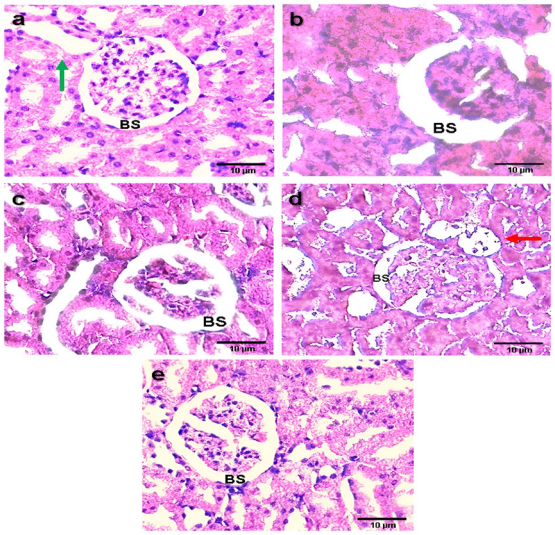


**Fig. 5: Light photomicrographs of glomeruli and renal tubules in the kidney of rats from different experimental groups (magnification 400X, H&E staining). (a) Normal control rats showing normal-sized glomeruli with well-defined renal tubules [indicated by the green arrow]. (b) STZ-induced diabetic rats depicting glomeruli shrinkage and large Bowman's space (BS). (c) Diabetic rats treated with 25 mg/kg bwt of lauric acid showed improvement in renal tubules and an increase in glomeruli size. (d) Diabetic rats treated with 50 mg/kg bwt of lauric acid revealed normal glomeruli size with tubular necrosis [indicated by red arrow]. (e) Diabetic rats treated with 100 mg/kg bwt of lauric acid displayed enlargement in glomeruli size better than the diabetic control group.**

## FTIR and chemometric analysis

To differentiate the treatment effects, kidney homogenates were subjected to FTIR spectroscopy. Fig. 6(a) shows the kidney FTIR absorption of the experimental groups in 4000-400 $cm^{-1}$ regions. We noticed that all kidney samples displayed an intense band at 1511-1545 $cm^{-1}$ (corresponding to amide II functional groups of proteins) and had a broad strong peak at the 3100-3600 $cm^{-1}$ region (arises from the hydroxyl group).

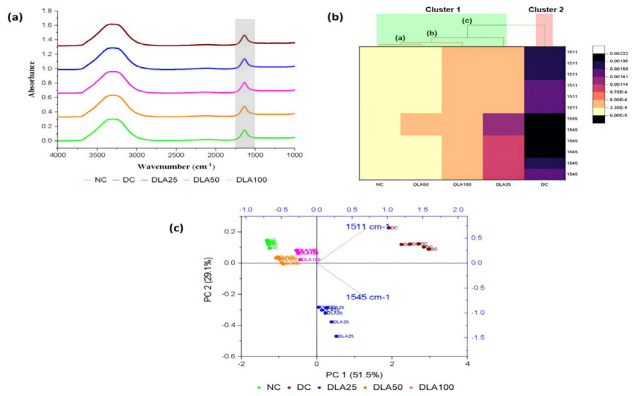


**Fig. 6: FTIR analysis of the rat kidney and chemometric analyses. (a) The average FTIR absorption spectra of kidney tissue in the regions of 4000-400 $cm^{-1}$. (b) Heat-map dendrogram of kidney IR intensities. Each column shows the peak intensities of individual animals from the experimental groups. A white and black indicate high and low concentrations of peak intensity, respectively. (c) PCA analysis represents the scores of samples (dots) and loading of variables (vectors) of second derivative spectra of kidney tissue on the first two principal components (80.6% of total variance). The analysis of these spectra was performed on n = 6 animals for each group.**

We further compare the peak intensities obtained from the integrated peak area of the selected frequency limits. Table I shows the peak intensity of the two IR features. The intensity of amide II peaks (1511 and 1545 $cm^{-1}$) was increased in the DC animals. However, the intensity of peaks decreased significantly in all treated groups. Importantly, no significant difference was observed between the DLA50 and NC groups at 1511 $cm^{-1}$, as indicated by their similar superscript notation in Table I.

**Table I: The intensity of potential IR marker bands in the kidney of experimental groups.**

| Parameters ($cm^{-1}$) | Frequency Limits for Integration ($cm^{-1}$) | Groups | | | | |
|---|---|---|---|---|---|---|
| | | NC | DC | DLA25 | DLA50 | DLA100 |
| **1545** | 1548 - 1539 (IPA X $10^{-3}$) | 0.127± 0.021$^{e}$ | 1.974± 0.213$^{a}$ | 1.108± 0.115$^{b}$ | 0.327± 0.040$^{d}$ | 0.541± 0.028$^{c}$ |
| **1511** | 1514-1504 (IPA X $10^{-3}$) | 0.070± 0.003$^{d}$ | 1.644± 0.146$^{a}$ | 0.520± 0.045$^{b}$ | 0.154± 0.021$^{d}$ | 0.370± 0.007$^{c}$ |

Values present as mean ± SD for six rats in each group. Values with different superscripts $^{a,b,c,d,e}$ in a row differed significantly at $p<0.05$ due to treatment effects. NC: normal control; DC: diabetic control; DLA25: diabetic treated with 25 mg/kg lauric acid; DLA50: diabetic treated with 50 mg/kg lauric acid and DLA100: diabetic treated with 100 mg/kg lauric acid

Two-way hierarchical clustering heat-map analysis (HCA) was employed to discriminate the dataset according to similarities. The HCA heatmap categorised animal groups into two main clusters based on the relative intensity of the IR features [Fig. 6(b)]. Cluster

1 consists of the NC, DLA25, DLA50, and DLA100 groups, while Cluster 2 consists of the DC animals. The PCA biplot identified peaks at 1511 and 1545 $cm^{-1}$ as kidney spectral markers with a total variance of 80.6%.

## DISCUSSION

The study demonstrated the nephroprotective activity of lauric acid against STZ-induced nephrotoxicity and renal dysfunction in rats. We showed that STZ-induced DN animals had survived with compromised kidney function and morphological irregularity changes such as mesangial expansion and nodular glomerulosclerosis that mimic those observed in humans. Increased serum creatinine, BUN, and MDA levels, as well as decreased SOD and CAT activities, strengthened the existence of kidney failure in STZ-induced diabetic rats. Our data also adds evidence for the potential of intraperitoneal injection of STZ at a dose of 40 mg/kg bwt to develop persistent DN in rats within 8 weeks [32].

A major finding from the present study is that the administration of lauric acid resulted in a significant increase in glucose tolerance and insulin concentrations across all groups. This finding is consistent with [33], who demonstrated that the combined intraduodenal administration of lauric acid and tryptophan led to increased insulin secretion. However, we observed herein that only animals in the DLA50 and DLA100 groups exhibited a significant reduction in FBG levels and their kidney weight. These findings align with previous studies showing a positive correlation between blood glucose level and kidney weight [34,35]. One possibility for these findings is that lauric acid supplementation at 50 and 100 mg/kg bwt may delay the progression of DN through its glucose-lowering effect in rats. Our data support previous research demonstrated that lauric acid delayed the rise in postprandial glucose by slowing gastric emptying [36].

To further determine the optimal dose of lauric acid against DN, biochemical analyses of serum and kidney homogenates were performed. We noticed that all doses of lauric acid administration resulted in significantly reduced MDA levels and increased activity of antioxidant enzymes (SOD and CAT) in the serum and kidney. Significantly lower serum creatinine and BUN levels were observed in all treated groups, strengthening the nephroprotective potential of lauric acid. It is worth noting that a high BUN-to-creatinine ratio indicates kidney dysfunction or damage [37]. Therefore, it is conceivable to suggest that lauric acid supplementation could improve kidney function in DN animals by enhancing insulin secretion and antioxidant defence. Namachivayam & Valsala Gopalakrishnan [38] has demonstrated that lauric acid enhances key endogenous antioxidant enzymes, including SOD, reduced glutathione peroxidase (GPx), and glutathione (GSH) in the liver of rats. While direct evidence regarding the effect of lauric acid on the kidney is limited, enhancing antioxidant enzyme activity is crucial for mitigating oxidative damage to renal cells, thereby preventing the progression of DN. The results were in agreement with [39,40], who demonstrate the nephroprotective activity of virgin coconut oil, which contains mostly lauric acid. A curious observation is that only the DLA50 animals had normal MDA values in serum and kidney. MDA is a biochemical indicator related to the degree of cellular damage [41]. This finding implies that the reduction in FBG values is influenced to some extent by the functional and histological recovery of the kidney. However, further studies are needed to understand the pathophysiological basis of this association and to determine whether pathological changes can differentiate the effectiveness of lauric acid in the treatment of DN.

Microscopic examination of tissue sections stained with H&E confirmed that the kidneys are vulnerable to STZ [42]. We noticed the improvement in kidney histology was consistent with the reduction in FBG and MDA levels. As shown in Fig. 5, only the DLA50 animals with the highest percentage of FBG reduction and insulin secretion and the lowest MDA values have normal-sized glomeruli. These findings were consistent with previous studies showing that tight glycaemic control by insulin therapy reduced glomerular tissue deterioration in animal models and humans [43,44]. Our observations also strongly support a reciprocal relationship between kidney and glucose homeostasis [45,46].

FTIR spectroscopy has potential as a diagnostic tool for monitoring glomerulosclerosis [47]. The strength of our study is that FTIR spectroscopy was employed to predict the spectral features associated with functional and histological changes in the kidneys following lauric acid treatment. As shown in Table I, the DC, DLA25, DLA50, and DLA100 groups displayed significantly higher peak intensities at 1511 and 1545 cm-1 than the NC group, supporting the incidence of DN in STZ-induced rats. It has been reported that changes in the peak intensity at 1500-1600 cm-1 were linked to transforming growth factor-β (TGF-β), a reliable biomarker for chronic kidney disease [48]. TGF-β may contribute to glomerulosclerosis by promoting the accumulation of extracellular matrix (ECM) proteins in the glomerulus and activating signalling pathways that lead to cell apoptosis [49]. More importantly, we noticed that a significant difference obtained at 1511 $cm^{-1}$ was consistent with our observations of MDA levels. No significant difference in peak intensity was observed between the NC and DLA50 groups that had normalized MDA values. Taken together, our findings suggest that lauric acid is effective at 50 mg/kg bwt in promoting functional and histological recoveries of kidneys in STZ-induced DN rats. This efficacy is supported by the observation that only this dosage demonstrated the ability to simultaneously modulate glycaemic control and normalise oxidative stress levels in the DN rats.

We also found that the FTIR peak around 1511 $cm^{-1}$ may serve as a promising spectral marker for the early detection of kidney injury.

## CONCLUSION

The present study showed that the optimal dose of lauric acid against STZ-induced DN in rats was 50 mg/kg bwt. Eight weeks of lauric acid supplementation attenuates structural and functional abnormalities in the kidney, in part by reducing blood glucose and improving antioxidant activity. We also suggest that FTIR spectroscopy combined with chemometric analysis is a reliable diagnostic tool for determining the degree of DN and distinguishing treatment effects.

## ACKNOWLEDGEMENT

The authors express their sincere appreciation to Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) for the facilities and technical support. This research was supported by grants from the industrial grants [MIH-(011/2020) 100-TNCPI/PRI 16/6/2 (028/2020)].

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