

Research Article

## Lauric Acid Alleviates Inflammation and Structural Changes in the Lungs of Type II Diabetic Male Wistar Rats

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**Keywords:**

Lauric acid, Type II diabetes, Hyperglycemia, Lungs, Pneumopathy, Leucocytes infiltration, Inflammation, Wistar rats

**ABSTRACT**

Lauric acid is a medium-chain fatty acid that has been reported to possess anti-inflammatory, antioxidant and antibacterial properties. Diabetic complication in the lungs is characterized by infiltration of inflammatory mediators and structural alteration of the lung parenchyma. This study was designed to evaluate the effect of lauric acid on leucocytes infiltration in bronchoalveolar lavage fluid (BALF), concentration of tumor necrosis factor- $\alpha$  and lung histology of type II diabetic male Wistar rats. A total of thirty-five male Wistar rats were randomly divided into seven groups of five rats each as follows: Group I served as normal control; group II were normoglycemic rats, administered 125 mg/Kg bwt lauric acid. Group III served as diabetic control. Groups IV, V, VI and VII were diabetic Wistar rats treated with 125 mg/Kg bwt, 250 mg/Kg bwt, 500 mg/Kg bwt lauric acid and 100 mg/Kg bwt metformin respectively. The results obtained, showed a significant ( $P \leq 0.05$ ) increase in total white blood cell count and differential count of lymphocytes, neutrophils and macrophages in blood and BALF of the diabetic control compared to the normal control. However, there was a significant decrease in total and differential white blood cell count in blood and BALF of the diabetic groups treated with lauric acid compared to the diabetic control ( $P \leq 0.05$ ). The concentration of TNF- $\alpha$  was significantly higher in the lungs of diabetic rats compared to the normal control, but the concentration was significantly reduced after treatment with lauric acid ( $P \leq 0.05$ ). Lauric acid also reversed the reduced alveolar spaces in diabetic lungs. These results indicate that lauric acid reduced inflammation and reversed the histoarchitectural alterations in the lungs of type II diabetic male Wistar rats.

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**INTRODUCTION**

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia caused by absolute or relative insulin deficiency, resulting in metabolic consequences of impaired carbohydrates, proteins and lipids metabolisms (Keerthi *et al.*, 2012). It is the most common of all endocrine diseases and is a threat to both young and old due to its increasing prevalence and associated disabling complications (IDF, 2017). Type II diabetes forms about 90% of all diabetes cases, making it the most common type and the main driver of the diabetes epidemic, affecting about 5.9% of the world's

adult population with almost 80% from developing countries (WHO, 2016).

Pulmonary complications of diabetes which are also called diabetic pneumopathy, have been reported for many decades, however, because the lung microvascular system has huge reserve function, diabetic lung damage is quite subclinical and often ignored (Kapoor *et al.*, 2015; Almeida *et al.*, 2016). The association between impaired lung function and diabetes is thought to be the result of biochemical changes in the structures of the lung tissue and airways that involves a series of mechanisms likely due to systemic inflammation, oxidative stress, hypoxemia and the direct damage caused by chronic hyperglycemia (Rogliani *et al.*, 2015). The impaired lung functions in patients with diabetes appears to be inversely related to blood glucose levels, duration of disease and its severity. A histological

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investigation of diabetic rabbit lungs revealed that, the diabetic lungs exhibited morphological abnormalities associated with an intense inflammatory reaction, characterized by massive inflammatory cell infiltration, within 3 weeks of diabetes induction (Zheng *et al.*, 2017). The structural changes in diabetic lungs have been described by Kuziemyky *et al.* (2011), as thickening of walls of pulmonary alveoli caused by elevated levels of collagen and elastin; thickening of a basal membrane of alveoli, and elastic recoil ability; thickening of basal membrane of capillaries and endothelium. Diabetes mellitus complication in the lungs is also associated with recruitment and infiltration of macrophages, neutrophils and other inflammatory cells into the lung tissues (Moral-Sanz *et al.*, 2012). The mechanism of cellular infiltration into the lungs in diabetes may be due to systemic inflammation induced by lipotoxicity, which triggered toll-like receptors activation on free fatty acid sensing (Lynch *et al.*, 2014). The infiltrating macrophages expresses peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ), and also produce inflammatory cytokines such as interleukin-1 $\beta$ , interleukin-6 and tumor necrosis factor- $\alpha$ , which play a role in mediating inflammatory responses (Cho *et al.*, 2014; Chen *et al.*, 2015).

Lauric acid is a saturated fatty acid that is found in many vegetable oils and fats, particularly in coconut oil, palm kernel oil and laurel oil. A significant component of saturated fatty acid found in human breast milk was reported to consist of lauric acid (Aly *et al.*, 2013). It is one of the most active ingredient of coconut oil, and composed of over 52% of the total 92% saturated fats present in coconut oil. It has 12 carbon atom chain, thus making it a medium chain fatty acid with a molecular formula: C<sub>12</sub>H<sub>24</sub>O<sub>2</sub> (Uday *et al.*, 2014; Anzaku *et al.*, 2017). Although a saturated fatty acid, lauric acid was reported to have decreased the mRNA expression of apolipoprotein, decrease low density lipoprotein (LDL) levels, but raise high density lipoprotein (HDL) levels, such that the total cholesterol level decline (Arunima and Rajamohan, 2018). Studies have shown that lauric acid was responsible for the pharmacological properties of coconut oil such as antioxidant (Alves *et al.*, 2017), anti-hyperlipidemic, anti-inflammatory, and antimicrobial properties (Dayrit, 2015). This study was therefore designed to evaluate the potential benefits of lauric acid on leucocytes infiltration in BALF, concentration of TNF- $\alpha$ , and lung histology of type II diabetic male Wistar rats.

## MATERIALS AND METHODS

### *Drugs and Reagents*

Lauric acid (CAS-NO:143-07-7) and Streptozotocin (STZ) were obtained from Sigma Aldrich (USA), Simas Margarine was purchased from PT Salim Ivomas

Pratama Tbk, (Indonesia), Rats Enzyme-linked immunosorbent assay (ELISA) kits TNF- $\alpha$  was purchased from Wuhan Fine Biotech Co. Ltd (China), Fructose (20% w/v) was purchased from Kem Light Laboratories PVT Ltd (India), Metformin was purchased from Jiangsu Ruinian Qianjin Pharmaceutical Co. Ltd (China). Other reagents used were of analytical grade and procured from appropriate manufacturing companies.

### *Experimental Design*

Thirty-five (35) apparently healthy male Wistar rats 7-8 weeks old, weighing 80-100g, were purchased from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The animals were housed in the Animal House of Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were provided with Grower's mash and water *ad libitum* and were acclimatized to the laboratory conditions for two weeks. The experimental protocol was reviewed and certified by Ahmadu Bello University committee on Animal Use and Care (ABUCAUC), with approval number (ABUCAUC/2018/071). Strict adherence to the Ethical Committee's directives was observed.

### *Preparation of High Fat Diet and Induction of Type II Diabetes Mellitus*

High fat diet (HFD) preparation and induction of type II diabetes were done according to the method of Okoduwa *et al.* (2017) with some modifications. Normal diet feed (NDF) containing: 18% Fats, 28% Proteins, 54% Carbohydrates, was mixed with Simas margarine: 99.9% Fats, groundnut mill: 46.2% Fats, 25.2% Proteins, 21.3% Carbohydrates and ground nut oil: 100% Fats. The HFD was constituted by mixing 10 grams of NDF with 2.5 grams Simas margarine, 2.5 grams of groundnut mill and 1 gram of ground nut oil. The nutrient composition of the prepared HFD was 41% Fats, 22% Proteins and 37% Carbohydrates. The animals were fed with the HFD along with 20% fructose solution as drinking water for six weeks, after which they were fasted overnight and injected intraperitoneally with a single, low dose of streptozotocin (STZ), at a dose of 40 mg/kg diluted in 0.1 M citrate-buffered saline (pH 4.5). The normal control rats were also injected with equal volumes of citrate-buffered saline. The rats were provided 5% glucose solution as drinking water in the first 24 hours after STZ injection. Diabetes was confirmed by determining blood glucose concentrations 72 hours after STZ administration, while the validation of diabetes was done one week after initial confirmation, and rats with fasting blood glucose levels  $\geq$  300 mg/dl were considered diabetic.

**Table 1:** Effect of Lauric Acid on the Mean Total and Differential White Blood Cell Counts in the Bronchoalveolar Lavage Fluid (BALF) of Type II Diabetic Male Wistar Rats.

Groups	WBC (X 10 <sup>9</sup> /L)	Lymphocytes (X 10 <sup>9</sup> /L)	Neutrophils (X 10 <sup>9</sup> /L)	Macrophages (X 10 <sup>9</sup> /L)
Normal control	1.53±0.23	0.92±0.21	0.54±0.06	0.05±0.01
NG+125 mg/Kg bwt LA	2.38±0.37	0.86±0.91 <sup>b</sup>	0.61±0.07 <sup>b</sup>	0.14±0.03 <sup>b</sup>
Diabetic control	4.25±0.39 <sup>a</sup>	2.07±0.27 <sup>a</sup>	1.61±0.30 <sup>a</sup>	0.51±0.09 <sup>a</sup>
DB+125 mg/Kg bwt LA	1.90±0.17 <sup>b</sup>	1.04±0.08 <sup>b</sup>	0.54±0.07 <sup>b</sup>	0.12±0.02 <sup>b</sup>
DB+250 mg/Kg bwt LA	3.60±1.06 <sup>a</sup>	1.00±0.25 <sup>b</sup>	0.91±0.24	0.25±0.04 <sup>ab</sup>
DB+500 mg/Kg bwt LA	1.53±0.16 <sup>b</sup>	0.70±0.05 <sup>b</sup>	0.40±0.05 <sup>b</sup>	0.11±0.01 <sup>b</sup>
DB+100 mg/Kg bwt Met	1.80±0.26 <sup>b</sup>	0.77±0.08 <sup>b</sup>	0.87±0.18	0.14±0.02 <sup>b</sup>

Results are presented as Mean ± Standard Error of Mean of five (5) rats. Values with superscript are significant at  $P \leq 0.05$ . a= When compared to the normal control, b= When compared to the diabetic control. NC= Normal control, NG= Normoglycemic, DW= Distilled water, DC=Diabetic control, TW=Tween 80, LA= lauric acid, DB= Diabetes, Met= Metformin

#### Animal Grouping and Treatment

After the induction of type II diabetes mellitus, the rats were divided into seven (7) groups of 5 rats each. Lauric acid was dissolved in tween 80 (vehicle) at a ratio of 1:2 (w/v) and diluted to desired concentrations in distilled water.

Group I (Normal control): Normoglycemic rats administered 1ml/Kg bwt distilled water orally, once daily for three weeks.

Group II: Normoglycemic rats administered 125 mg/Kg bwt Lauric acid orally, once daily for three weeks.

Group III (Diabetic control): Diabetic rats administered 1ml/Kg bwt tween 80 orally, once daily for three weeks.

Group IV: Diabetic rats administered 125 mg/Kg bwt of Lauric acid orally, once daily for three weeks.

Group V: Diabetic rats administered 250 mg/Kg bwt of Lauric acid orally, once daily for three weeks.

Group VI: Diabetic rats administered 500 mg/Kg bwt of Lauric acid orally, once daily for three weeks.

Group VII: Diabetic rats administered 100 mg/Kg bwt Metformin, orally, once daily for three weeks

#### Determination of Blood Glucose Level

Blood was obtained from tail vein, and blood glucose level was determined using glucose test strips and digital glucometer based on glucose oxidase principle (Rheney and Kirk, 2000).

#### Sample Collection

At the end of the three (3) weeks treatment period, the rats were fasted overnight, and then anaesthetized with 50mg/Kg Ketamine hydrochloride. Blood samples were then collected via cardiac puncture using 5ml syringes and were stored in EDTA bottles which was used for total and differential white blood cell count.

#### Collection of Broncho Alveolar Fluid (BALF)

Broncho Alveolar lavage procedure was performed surgically in an anesthetized rat. 2ml of normal saline was infused into the lungs through the trachea by means of a cannula. The fluid was rinsed and aspirated 30 seconds later. The procedure was repeated, and the collected fluid was centrifuged at 3000 x g for 10 minutes. The supernatant was used for total and differential white blood cell count (Chaudhari *et al.*, 2011).

#### Preparation of Lung Homogenate

Lung homogenate was prepared from the left lung. The lung was removed, weighed and homogenized with phosphate buffered saline (140 mM KCl, 20 mM phosphate, pH 7.4). Every one gram of lung tissue was homogenized with 9ml of phosphate buffer. The homogenate was centrifuged at 9000 x g for 30 min and the supernatant was used for measurement of TNF- $\alpha$  concentration (Samarghandian *et al.*, 2014).

#### Hematological Analysis

Total white blood cell counts in blood and BALF were determined using hemocytometric method and were counted using light microscope with X10 magnification while the differential white blood cells were counted using a magnification of X100 (Cheesbrough, 1999).

#### Biochemical Assay of TNF- $\alpha$

The assay for the tumor necrosis factor-alpha concentration, was carried out using rat TNF- $\alpha$  ELISA Kit according to manufacturer's instruction.

#### Histological Studies of Lung Tissue

The right lungs were used for histological studies, using H and E staining technique as described by Bancroft and Stevens (1990). The slides were examined with light

microscope at a magnification of X100 and photomicrographs taken.

#### Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. Data are expressed as mean  $\pm$  Standard Error of Mean (SEM). Statistical significance of differences was assessed using one-way analysis of variance (ANOVA), followed by a Tukey's *post-hoc* test for multiple comparisons between groups. Values of  $P \leq 0.05$  were considered statistically significant.

## RESULTS

Table 1 shows the effect of lauric acid on the total and differential white blood cell count in BALF of diabetic rats. There was a significant increase in total WBC and differential count of lymphocytes, neutrophils and macrophages in the diabetic control compared to their respective counts in the normal control ( $P \leq 0.05$ ). However, after treatment of the diabetic rats with 125 mg/Kg bwt, 250 mg/Kg bwt and 500 mg/Kg bwt lauric acid as well 100 mg/Kg bwt Metformin, there was a significant decrease in total WBC and differential counts

**Table 2:** Effect of Lauric Acid on the Mean Total and Differential White Blood Cell Counts in the Blood of Type II Diabetic Male Wistar Rats.

Groups	WBC ( $\times 10^9/L$ )	Lymphocytes ( $\times 10^9/L$ )	Neutrophils ( $\times 10^9/L$ )	Macrophages ( $\times 10^9/L$ )
Normal control	1.53 $\pm$ 0.23	0.92 $\pm$ 0.21	0.54 $\pm$ 0.06	0.05 $\pm$ 0.01
NG+125 mg/Kg bwt LA	2.38 $\pm$ 0.37	0.86 $\pm$ 0.91 <sup>b</sup>	0.61 $\pm$ 0.07 <sup>b</sup>	0.14 $\pm$ 0.03 <sup>b</sup>
Diabetic control	4.25 $\pm$ 0.39 <sup>a</sup>	2.07 $\pm$ 0.27 <sup>a</sup>	1.61 $\pm$ 0.30 <sup>a</sup>	0.51 $\pm$ 0.09 <sup>a</sup>
DB+125 mg/Kg bwt LA	1.90 $\pm$ 0.17 <sup>b</sup>	1.04 $\pm$ 0.08 <sup>b</sup>	0.54 $\pm$ 0.07 <sup>b</sup>	0.12 $\pm$ 0.02 <sup>b</sup>
DB+250 mg/Kg bwt LA	3.60 $\pm$ 1.06 <sup>a</sup>	1.00 $\pm$ 0.25 <sup>b</sup>	0.91 $\pm$ 0.24	0.25 $\pm$ 0.04 <sup>ab</sup>
DB+500 mg/Kg bwt LA	1.53 $\pm$ 0.16 <sup>b</sup>	0.70 $\pm$ 0.05 <sup>b</sup>	0.40 $\pm$ 0.05 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>b</sup>
DB+100 mg/Kg bwt Met	1.80 $\pm$ 0.26 <sup>b</sup>	0.77 $\pm$ 0.08 <sup>b</sup>	0.87 $\pm$ 0.18	0.14 $\pm$ 0.02 <sup>b</sup>

Results are presented as Mean  $\pm$  Standard Error of Mean of five (5) rats. Values with superscript are significant at  $P \leq 0.05$ . a= When compared to the normal control, b= When compared to the diabetic control. NC= Normal control, NG= Normoglycemic, DW= Distilled water, DC=Diabetic control, TW=Tween 80, LA= lauric acid, DB= Diabetes, Met= Metformin

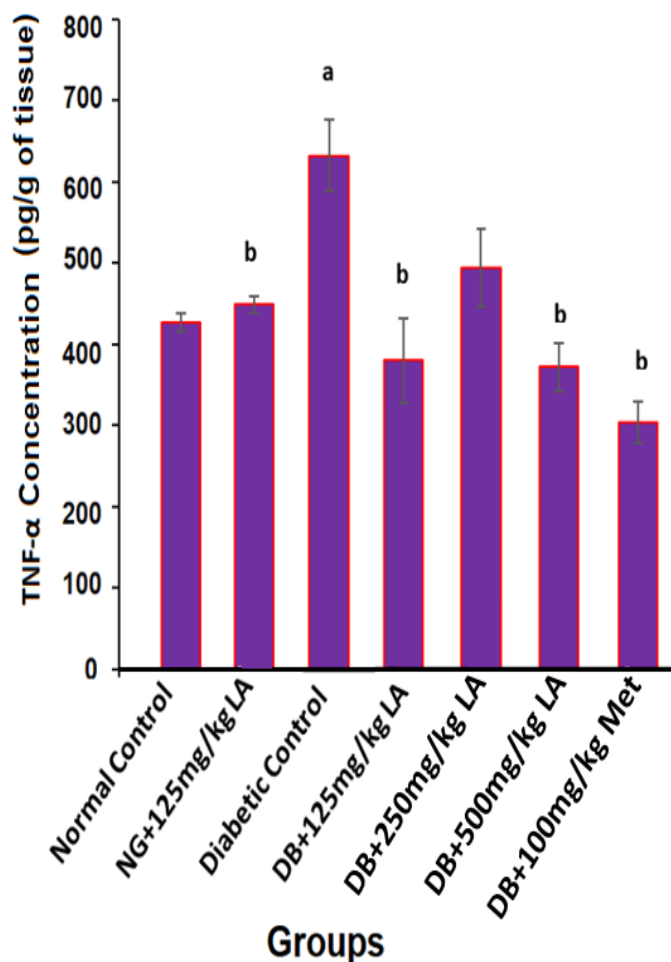
when compared to diabetic control ( $P \leq 0.05$ ), except for 250 mg/Kg bwt Lauric acid and 100 mg/Kg bwt Metformin treated groups which did not significantly reduce the differential count of neutrophils and in addition, 250 mg/Kg bwt Lauric acid did not also significantly reduce the total WBC count compared to diabetic control ( $P > 0.05$ ). The normoglycemic rats treated with 125 mg/Kg bwt lauric acid, did not show any significant difference for total WBC, lymphocytes, neutrophils and macrophages counts when compared to the normal control ( $P > 0.05$ ), however, there was a significant decrease in lymphocytes, neutrophils and macrophages counts when compared to their respective counts in the diabetic control ( $P \leq 0.05$ ).

In Table 2, the effect of lauric acid on the mean total and differential white blood cell count in the blood of type II diabetic male Wistar rats shows that the mean total WBC count in the diabetic control was significantly increased when compared to normal control ( $P \leq 0.05$ ). The total WBC and differential count of lymphocytes, neutrophils and monocytes of normoglycemic rats treated with 125 mg/Kg bwt lauric acid, diabetic rats treated with 125 mg/Kg bwt and 500 mg/Kg bwt lauric acid, were significantly reduced when compared to the diabetic control ( $P \leq 0.05$ ), except for diabetic rats treated with 125mg/Kg bwt lauric acid which did not show

significant difference in differential count of lymphocytes compared to diabetic control ( $P > 0.05$ ). The mean total and differential WBC counts in diabetic rats treated with 250 mg/kg lauric acid showed no significant difference when compared to diabetic control ( $P > 0.05$ ). Diabetic rats treated with 100 mg/Kg bwt Metformin only showed significant decrease in differential count of neutrophils and monocytes when compared with diabetic control ( $P \leq 0.05$ ).

The result in Figure 1 shows the effect of lauric acid on the mean concentration of TNF- $\alpha$  in the lungs of type II diabetic male Wistar rats. The concentration of TNF- $\alpha$  in diabetic control was significantly increased at  $P \leq 0.05$  compared to the normal control. Normoglycemic rats group treated with 125 mg/Kg bwt lauric acid has a significantly lower concentration of TNF- $\alpha$  when compared with diabetic control ( $P \leq 0.05$ ). However, it did not show significant difference when compared with normal control ( $P > 0.05$ ). There was a significant decrease in the concentrations of TNF- $\alpha$  in the diabetic rats treated with 125 mg/Kg bwt, 500 mg/Kg bwt lauric acid and 100 mg/Kg bwt metformin when compared with diabetic control ( $P \leq 0.05$ ). The concentration of TNF- $\alpha$  in the diabetic rats treated with 250 mg/Kg bwt lauric acid did not show significant difference when compared to diabetic control ( $P > 0.05$ ).

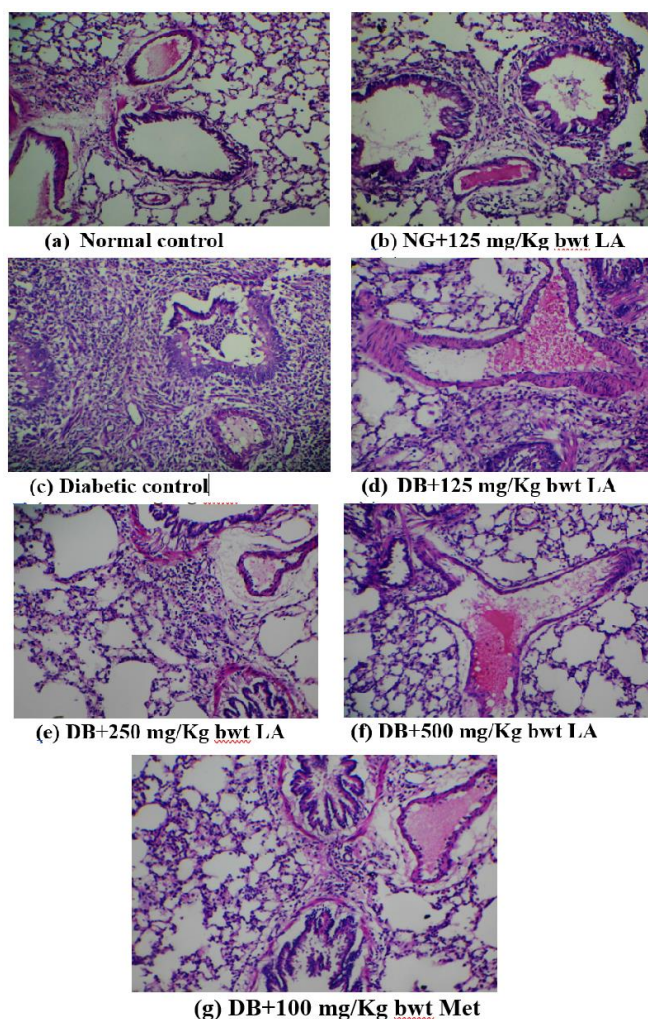




**Fig.1:** Effect of Lauric Acid on the Mean Concentration of Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) in the Lungs of Type II Diabetic Male Wistar Rats. Results are presented as Mean  $\pm$  Standard Error of Mean of five (5) rats. Values with superscript are significant at  $P \leq 0.05$ . a= When compared to the normal control, b= When compared to the diabetic control. NC= Normal control, NG= Normoglycemic, DW= Distilled water, DC=Diabetic control, TW=Tween 80, LA= lauric acid, DB= Diabetes, Met= Metformin

Fig. 2 shows the lung photomicrograph of the normal control and normoglycemic rats treated with 125mg/Kg bwt lauric acid, showing normal alveolar sac with no cellular infiltration and no significant pathological changes in the bronchial epithelium. Although, the lungs of normoglycemic rats treated with 125 mg/Kg bwt lauric acid showed mild cellular infiltration. There were obvious histopathological changes in the lungs of the diabetic control rats, characterized by thickened alveolar basement membrane, massive cellular infiltration and distorted bronchial epithelium. Treatment of diabetic rats with 125 mg/kg bwt, 250 mg/kg bwt and 500 mg/kg bwt lauric acid reduced the cellular infiltration in the lungs, and replacement with normal alveolar sac and bronchial epithelium. However, diabetic rats treated with 125 mg/Kg bwt lauric acid showed mild cellular infiltration in the lungs with congestion, but no obvious

pathological changes. Lungs of diabetic rats treated with 100 mg/Kg bwt metformin had a normal bronchial epithelium and alveolar sac but showed mild cellular infiltration.



**Fig. 2:** Photomicrographs of lung tissues of Normoglycemic/Diabetic Wistar rats Treated with Lauric Acid. (H and E, Magnification X 100). (a) Normal control rats with normal alveolar spaces without cellular infiltration. (b) NG+125 mg/Kg bwt LA: Normoglycemic rats treated with 125mg/Kg bwt lauric acid histological section shows normal alveolar spaces but with mild cellular infiltration. (c) Diabetic control shows massive cellular infiltration and distorted bronchial epithelium with thickened alveolar basement membrane. (d) DB+125 mg/Kg bwt LA: Diabetic rats treated with 125mg/Kg bwt lauric acid, Mild cellular infiltration with lungs with congestion, but no obvious pathological changes in the alveolar. (e) DB+250 mg/Kg bwt LA: Diabetic rats treated with 250mg/Kg bwt lauric acid, Mild cellular infiltration with normal alveolar spaces. (f) DB+500 mg/Kg bwt LA: Diabetic rats treated with 500mg/Kg bwt lauric acid, Mild cellular infiltration with normal alveolar spaces. (g) DB+100mg/Kg bwt Met: Diabetic rats treated with 100mg/Kg bwt lauric acid, Mild cellular infiltration with normal alveolar spaces.

## DISCUSSION

Diabetic pulmonary complication termed ‘diabetic pneumopathy’, is a glucotoxicity-induced complication

caused by chronic hyperglycemia. This complication leads to a reduction in lung function that is characterized by systemic inflammation-associated recruitment and infiltration of inflammatory cells and proinflammatory cytokines in the lung tissues (Niazi *et al.*, 2013). This study evaluated the effect of lauric on leucocytes infiltration in BALF, concentration of TNF- $\alpha$  and lung histology of type II diabetic male Wistar rats.

In the present study, it was observed that high fat diet-streptozotocin-induced type II diabetes mellitus caused a significant cellular infiltration into the lung tissues. This was characterized by increase in total WBC, lymphocytes, neutrophils and macrophages counts BALF as well as the blood of the diabetic rats. This result is consistent with the findings of a study by Neto *et al.* (2013), who reported increased pulmonary microvascular permeability and inflammatory cell infiltration within pulmonary tissues in diabetic rats. The increased differential and total WBC count in BALF may be secondary to the increase in the blood as seen in this study. Pulmonary alveolar macrophages (PAMs), derived from circulating monocytes, are the major resident phagocytic cells in the lungs. Together with neutrophils, they mediate first line of defense, as such, their number is usually increased in diabetes-associated lung inflammation. The PAMs exert their phagocytic activity in the lungs, through a mechanism related to the activation of Fc gamma receptors (Cheung *et al.*, 2000). Once activated, PAMs initiate inflammatory responses and recruit activated neutrophils into the alveolar spaces, and also release reactive intermediates such as superoxide ions and proinflammatory cytokines which play a significant role in the pathogenesis of immune complex mediated lung injury (Rubins, 2003). However, contrary to this result, Alba-Loureira *et al.* (2007), reported that alloxan-induced diabetes is associated with reduced number of leucocyte count, impaired inflammatory processes such as chemotaxis, phagocytosis and generation of superoxide ions by both neutrophils and macrophages. Besides macrophages and neutrophils, lymphocyte count was also increased in both BALF and blood of diabetic rats. Lymphocytes, particularly the CD4<sup>+</sup> have been reported to be recruited to the lungs in response to pulmonary infection (Xia *et al.*, 2017).

Treatment of diabetic rats with lauric acid in this study, significantly decreased the number of total and differential leucocytes count in the blood, and inhibited their infiltration into the lungs. Lauric acid was reported to inhibit the up-regulatory effect of interferon gamma (IFN- $\gamma$ ), which is a T-helper cell 1 produced cytokine that serves as a potent activator of macrophages during the innate and acquired immune response (Lim *et al.*, 2015). Therefore, reduction in IFN- $\gamma$  may consequently cause a decrease in the number of macrophages that infiltrate the lungs, as shown in this study. Lauric acid

has also been shown to possess antimicrobial activity, and has been reported to be effective in relieving inflammation caused by bacteria, viruses and fungi through inhibition of infiltration of neutrophils and macrophage inflammatory protein-2 to the site of the inflammation, as well as inhibition of TLR2 mediated production of pro-inflammatory cytokines (Nakatsuji *et al.*, 2009; Anzaku *et al.*, 2017). Lauric acid did not significantly affect the total and differential leucocyte count of lymphocytes, neutrophils and macrophages in the lungs of normoglycemic rats compared to the normal control. This implies that, the ability of lauric acid to exert its anti-inflammatory action by decreasing the number of total and differential white blood cells infiltrating the diabetic lungs, may be dependent on the existence of lung inflammation. Despite the fact that all the three doses of lauric acid reduced the infiltration of inflammatory cells into the lungs, the higher dose of 500 mg/Kg was shown to be the most effective while 250 mg/Kg dose the least effective. This occurred in spite of the fact that, all the three doses significantly decreased the mean fasting blood glucose level of diabetic rats in the same degree as metformin, a standard antidiabetic agent. Because metformin decreased the total and differential white blood cells count in blood and also inhibited their infiltration into the lungs, it implies that, lauric acid might have mediated its action of decreasing the number of infiltrating leucocytes, through a mechanism that may be related to its blood glucose lowering action.

This study also showed an increase in the concentration of tumor necrosis factor -alpha (TNF- $\alpha$ ), in the lungs of diabetic rats. The result is substantiated by the findings of studies conducted by Eren *et al.* (2010), and Zhang *et al.* (2015), who also reported an elevation in the concentration of TNF- $\alpha$  in the lungs of diabetic rats. Tumor necrosis factor- $\alpha$  is a proinflammatory cytokine, that at higher concentration leads to exacerbation of inflammatory and prooxidative responses, that are important in the pathogenesis of various pulmonary disorders (Patel *et al.*, 2013). Tumor necrosis factor- $\alpha$  mediated inflammatory responses are activated via Nuclear factor kappa B (NF- $\kappa$ B) signaling pathways, which play an important role in broad spectrum of inflammatory networks that regulate cytokine activity in the lungs, activated by toll-like receptors 2 and 4 subtypes (TLR2 and TLR4) (Dasu *et al.*, 2012; Schuliga, 2015). The TNF- $\alpha$  released from this inflammatory pathway, plays a crucial role in triggering alveolar epithelial dysfunction, leading to pulmonary edema, through activation of its p55 receptor-mediated death signaling involving caspase-8 (Patel *et al.*, 2013).

Lauric acid treated diabetic rats, showed a significant reduction in the concentration of TNF- $\alpha$  in their lung tissues. In agreement with this result, Nishimura *et al.* (2018), reported that lauric acid

decreased TLR4-mediated production of proinflammatory cytokines, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, associated with neuroinflammatory responses by activated microglia, via GPR40-dependent signaling cascade. However, contrary to the result of this study, Wang *et al.* (2018), reported that lauric acid activates TLR4 to facilitate the recruitment of myeloid differentiation primary response 88 (MyD88) and eventually promote nuclear translocation of NF- $\kappa$ B, leading to the expression of inflammatory genes that mediate inflammatory responses. Nuclear factor kappa B pathway and the mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) cascades, have been proposed as the two major mechanisms for modulation of the production of pro-inflammatory molecules like TNF- $\alpha$  and IL-1 $\beta$  (Huang *et al.*, 2014). These pathways which are prominent contributors to chronic inflammatory responses, have been shown to be suppressed by treatment with lauric acid.

The mechanism of anti-inflammatory action of lauric acid on TNF- $\alpha$  concentration in the diabetic lungs may be related to the activation of peroxisomal proliferator activated-receptor gamma (PPAR $\gamma$ ), which is a member of the nuclear hormone receptor superfamily that plays an important role in the regulation of inflammatory and immune reactions. Lauric acid may through PPAR $\gamma$ , protects against inflammation by its ability to mediate suppression of TLR activation, pro-inflammatory transcription factor, NF- $\kappa$ B activation and inhibition of pro-inflammatory mediators like TNF- $\alpha$ , while increasing the production of anti-inflammatory cytokines such as IL-4 and IL-10 (Dasu *et al.*, 2012; Kortekaas *et al.*, 2013). A study by Liberato *et al.* (2012), reported lauric acid as a partial agonist of PPAR- $\gamma$ , and may through its binding to PPAR- $\gamma$ , decrease the concentration of TNF- $\alpha$  in diabetic lungs, by inhibiting the expression of TLR and NF- $\kappa$ B inflammatory signaling pathways. The upregulation of PPAR- $\gamma$  was reported to cause an increased expression of interleukin-10 (IL-10), an anti-inflammatory cytokine that suppresses the level of proinflammatory cytokine TNF- $\alpha$  and interleukin-6 (Munoz and Costa, 2013). This may be another mechanism via which lauric acid exerts its anti-inflammatory action. Lauric acid at lower dose of 125 mg/Kg bwt and higher dose of 500 mg/Kg bwt and metformin at 100 mg/Kg bwt were shown to significantly reduced the level of TNF- $\alpha$  in the diabetic lungs, unlike 250mg/kg, lauric acid which did not significantly reduce the level of TNF- $\alpha$  in the diabetic lungs.

In this study, and as in a previous study by Suarez *et al.* (2016), the histology of the diabetic lungs, showed the presence of inflammatory cells, increased thickness of alveolocapillary basement membrane and decreased alveolar surface area. The mechanism of diabetic lung

injury was hypothesized to be hyperglycemia-induced upregulation of a variety of pathways including; NF- $\kappa$ B inflammatory pathway, oxidative stress and non-enzymatic glycosylation of proteins of the lungs (Zheng *et al.*, 2017). Excessive non-enzymatic glycosylation of proteins of the lungs, impaired the cross-links of collagen and elastin, thus, decreasing both their strength and elasticity Suarez *et al.*, 2016). Increased thickness of the alveolo-capillary barrier and the expansion of the interstitium are associated with a reduction of alveolar space and narrowing of the pulmonary capillary network. These alterations contribute to a reduction in gas diffusion velocity through the alveolo-capillary membrane (Pitocco *et al.*, 2012). Treatment with lauric acid was shown to significantly reduce the level of infiltration of inflammatory cells into the lung tissues and also reduced the thickening of the alveolo-capillary basement membrane. The mechanism by which lauric acid was able to alleviate diabetic lung injury, may be related to its ability to exert anti-inflammatory action against both inflammatory cells and pro-inflammatory cytokines.

## CONCLUSION

Lauric acid exerts potent anti-inflammatory properties that are effective in ameliorating biochemical and structural changes in the lungs induced by type II diabetes mellitus.

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