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Research Article

Antidiabetic activity and kidney protection effect of lemon pepper nano-emulsion in streptozotocin-induced diabetic male Wistar rats

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Abstract
Some previous studies have looked for natural products to become supplementary for antidiabetic drugs when the diabetic case rate was high. Lemon pepper has been widely studied for antidiabetic activity. However, none of these studies developed other pharmaceutical forms of lemon pepper fruit. Due to this reason, this study aimed to investigate the antidiabetic activity of lemon pepper fruit in a nano-emulsion form. This study underwent an acute toxicity assay for lemon pepper nano-emulsion. Acute toxicity testing confirmed its safety, and an experimental study was conducted using twenty-five streptozotocin-induced diabetic rats divided into five groups: three treatment groups (25, 50, and 75 mg/kg body weight (BW) and pair of control (0.5% Sodium Carboxy Methyl Cellulose) and standard (100 mg/kg BW metformin) groups. On the other hand, this study also used five non-diabetic rats as a normal group. Five non-diabetic rats served as a normal group. Blood glucose levels were measured every five days, and kidney function tests (KFT) were performed on 15th day. All doses of lemon pepper nano-emulsion significantly reduced blood glucose levels by 71 mg/dL on the 10th day (P-Value < 0.05) and 89 mg/dL on the 15th day (P-Value: 0.001). Creatinine levels did not affect(P: Value: 0.233), but 50 mg/ kg BW of lemon pepper nano-emulsion significantly reduced blood urea nitrogen (BUN) levels to 40 mg/dL, with the highest dose (75 mg/kg BW) further lowering BUN to 38 mg/dL (P-Value: 0.001). In conclusion, lemon pepper nano-emulsion effectively decreased blood glucose level and mitigated kidney from hyperglycaemic-induced injury, highlighting the potential of its novel nano-formulation as a therapeutic option for diabetes management.

Keywords: Blood glucose, Kidney function, Lemon pepper, Nano-emulsion, Streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic metabolism disorder of carbohydrate, lipid, or protein with some causes, leading to increased blood glucose levels and occupying insulin function insufficiency. This insufficiency is caused by either absolute insulin deficiency from pancreatic tissue or insulin resistance (Kementrian Kesehatan Republik Indonesia, 2019). American Diabetic Association (ADA) classified it into several type, one of them is Non-Insulin Dependent Diabetes Mellitus (NIDDM), also known as Type 2 Diabetes Mellitus (Khan and Khan, 2017). NIDDM is a condition when the body is unable to utilise insulin hormone and lead

to hyperglycemic condition, followed by worsening lipid profile serum (Dharma Yudha *et al*., 2022).

Diabetes Mellitus is not only a global health burden but also a health burden in Asian Region. International Diabetic Federation (IDF) estimated that diabetic globally placed top ten causes of mortality, which was around 6.7 million deaths every year in people aged of 20 to 79 (Internation Diabetes Federation, 2019) Indonesia is seventh-ranked from ten countries with a high rate of diabetes mellitus cases, estimated at 10.7 million (Kementrian Kesehatan Republik Indonesia, 2020). Diabetes mellitus can cause various complications and increase medication costs (Arjani, 2018). Thus, looking for alternative treatments from natural products with minimal adverse drug effects and lower costs is important. One of these is lemon pepper, which is an indigenous herb from Indonesia.

Lemon pepper has the scientific name of *Zanthoxylum acanthopodium* DC particularly growing in North Sumatera Province, especially Toba Samosir and Tapanuli Utara region (Asbur and Khairunnisyah, 2018). Traditionally, lemon pepper is called as “Andaliman” in local citizens and is widely used as a food seasoning. However, some local ancestors also used either part of lemon pepper plants for various medication purposes (Al, 2019). Some previous studies have widely investigated its phytochemical contents and pharmacology effects. Saragih *et al*. (2019) reported the phytochemicals from lemon pepper were phenol, saponin, flavonoid, tannin, triterpenoid, and alkaloid. Flavonoid from lemon pepper has been reported to has antioxidant activity and α-glucosidase enzyme inhibitor from *in vitro* study. On the other hand, other phytochemical, saponin, was also reported to have regenerative effect for pancreatic beta cells in the pancreatic tissue that increased insulin secretion (Worotikan *et al*., 2017).

Some previous studies demonstrated antidiabetic activity from lemon pepper fruit in both *in vitro* and *in vivo* studies. Both lemon pepper ethanol and methanol extract have been reported to have antidiabetic activity. Moreover, lemon pepper ethanol extract also has liver protection activity in Non-Faty Liver Disease that was induced by diabetic conditions; meanwhile, lemon pepper methanol extract has a kidney protection effect against diabetic nephropathy (Chiuman *et al*., 2021, 2022)

Based on the information above, it becomes important to investigate the antidiabetic effect of lemon pepper extract as another pharmaceutical form, nano-emulsion, instead of oral suspension from Sodium Carboxy Methyl Cellulose (SCMC). Nano-emulsion as part of the nanoparticle is defined as an emulsion with a particle size range within 1 to 100 nanometers. Nano-emulsion has been reported to improve drug delivery by increasing the surface area (Mamillapalli *et al*., 2016). However, this study still used a streptozotocin-induced diabetic rat model, as described earlier by Chiuman *et al*. (2021, 2022). Hence, this study aimed to investigate an antidiabetic effect of lemon pepper nano-emulsion on the streptozotocin-induced diabetic rat model.

MATERIALS AND METHODS

Study Design

This experimental study was performed in the Pharmacology Laboratory, Universitas Prima Indonesia on October 2023-December 2023. This study has also been approved by Komite Etik Penelitian Kesehatan Universitas Prima Indonesia.

Materials

The materials used in this study included Lemon peppers, methanol solvent, methyl paraben, propyl paraben, distilled water, Tween 80, PEG400, Sodium Carboxyl Methyl Cellulose (SCMC), metformin tablet, streptozotocin powder, buffer citrate solution, normal saline, 10% buffer formalin solution, Haematoxylin and Eosin Powder, and Kidney Function Test (KFT).

Sample Collection and Extraction

The identified lemon pepper was extracted using the maceration method with methanol solvent. All lemon peppers were initially cleaned and dried in an oven at 55^{o}C for 5 hours. After that, it was meshed into lemon pepper powder by no. 40 Mesh sieve. Then, this lemon pepper powder was soaked in methanol solvent with a ratio of 1:6 for 24 hours and filtered it. Finally, the filtrate from filtration was evaporated by a rotary evaporator to obtain a concentrated extract.

Phytochemical screening

Concentrated lemon pepper methanol extract underwent phytochemicals screening to look for flavonoid, alkaloid, terpenoid, steroid, tannin, saponin, glycoside, and anthocyanin (Saragih *et al*. 2019; Suhartomi *et al*. 2020; Gulo *et al*., 2021; Chiuman *et al*., 2023).

Formulation of lemon pepper nano-emulsion

The concentrated lemon pepper extract was formulated into lemon pepper nano-emulsion. Initially, both 0.2 grams of methyl paraben and 0.1 grams of propyl paraben were dissolved into 50 ml of warm distilled water and chilled it. Then, thirty grams of tween 80 was gradually added to 50 ml of chilled distilled water, stirred for 30 minutes by a magnetic stirrer at a speed of 5,000 RPM, and labelled as the first mixture. After that, fifteen grams of PEG400 was mixed with five grams of lemon pepper by magnetic stirrer for 20 minutes at a speed of 5,000 RPM and labelled as a second mixture. Both the first and second mixtures were gradually mixed by a

magnetic stirrer at a speed of 5,000 RPM for 8 hours and underwent sonification.

Formulation of both control and standard suspension

The control and standard in this study were Sodium Carboxyl Methyl Cellulose (SCMC) and Metformin, respectively. This study used 0.5% SCMC, which was formulated by dissolving 0.5 grams of SCMC powder into a hundred millilitre of warm distilled water. Meanwhile, this SCMC was used as a control and dispersion medium for metformin. Five hundred Metformin tablets were ground into powder and suspended into twenty-five millilitres of 0.5% SCMC, and the metformin dose used in this study was 100 mg/ kg BW (5 ml/ kg BW).

Acute toxicity assay

A preliminary study, an acute toxicity assay, prior to an *in vivo* antidiabetic and kidney protection study aimed to determine the variation in doses. This study was used five rat, that had been acclimatized for 14 days yet. Acute toxicity assay was performed based on OECD (2001) Guidelines Number 401 using Fixed dose Protocols. These rat received five doses, including 2,000 mg/ kg body weight (BW), 1,000 mg/ kg BW, 500 mg/ kg BW, 150 mg/ kg BW, and 50 mg/ kg BW. Acute toxicity assay observed some acute toxicity signs, including locomotory activity, lethargy, weakness, distress, and sudden death.

Treatment

All male Wistar rats used in this study initially acclimatized for 14 weeks before receiving any treatment. Diabetic condition in this study was induced by streptozotocin solution. Amount of 50 mg of streptozotocin powder was dissolved into 10 ml of buffer citrate solution (pH: 4). After that, it was injected intraperitoneally in a dose of 45 mg/ kg BW and diabetic rats was defined as a blood glucose level higher than 200 mg/ kg BW.

Antidiabetic assay used thirty male Wistar Rats, which were grouped into six groups: normal, control, standard, and Lemon Pepper Nano-Emulsion 1, 2, and 3. The Normal group did not receive any treatment nor streptozotocin diabetic induction. Meanwhile, control and standard group members of diabetic rat received a millilitre of 0.5% SCMC suspension and 100 mg/ kg BW of metformin suspension, respectively. Moreover, lemon pepper nano-emulsion groups were diabetic rats that received lemon pepper nano-emulsion according to a toxic dose. The lowest dose of lemon pepper nano-emulsion that did not show any toxicity dose was administrated for Lemon Pepper Nano-Emulsion-2. On the other hand, half a dose of Lemon Pepper Nano-Emulsion 2 was administrated for Lemon Pepper Nano-Emulsion 1 and the last was Lemon Pepper Nano-Emulsion 3 received one-third dose of Lemon Pepper Nano-Emulsion 1. All these treatments were administrated once a day for two weeks.

Blood glucose level and kidney function test (KFT)

Blood glucose level in this study was measured by glucometer, that was used vein blood from the tail vein. Blood glucose level was expressed as mg/ dl. All rats in this study regularly underwent blood glucose level measurement, including before and after streptozotocin induction on the 5^{th}, 10^{th}, and 15^{th} day.

After two weeks, exactly on the fifteenth day, all rats were sacrificed by ketamine injection. Then, these rats were fixed into paraffin block. After that, these rats were vertically incised in the abdomen and thorax. The blood was withdrawn by intracardiac puncture, which was undergone by 23G needle and five five-millilitre syringe. Obtained blood was collected into a red-coloured blood tube.

Kidney Function Test included blood urea nitrogen and creatinine serum level. Prior to KFT test, the obtained blood was initially centrifuged at 3,000 RPM for 30 minutes. After that, this blood was separated into serum in the upper layer and blood clot in the lower layer. The serum was used to measure the blood urea nitrogen and creatinine based on Medan Regional Health Laboratory (Laboratorium Kesehatan Daerah).

Animal ethical approval

The use of animals in this study has been approved by the Health Research Ethics Committee of Universitas Prima Indonesia (Approval No. 001/KEPK/UNPRI/IV/2022) in accordance with the Seven Principles of the World Health Organization (WHO) (2011) guidelines.

Data analysis

All data were analysed by IBM SPSS 26. Initially, all data was analysed using descriptive statistics, which described all data as a narration and table. Blood glucose level and KFT test were then analysed by inferential statistics. Preceding inferential statistics, the data distribution was analysed by Shapiro-Wilk. If data distribution was normal, then it was analysed using a parametric statistic called one-way ANOVA. If Elseif data distribution was not normal, it was analysed using non-parametric statistics, which was Kruskal-Wallis.

RESULTS AND DISCUSSION

Physical characteristic of lemon pepper methanol extract

This study used lemon pepper from a traditional market that was extracted by maceration methods and the physical characteristics of lemon pepper methanol ex-

Table 1. Physical Characteristics of lemon pepper methanol extract

Physical Characteristic	Value
Raw Material Mass (grams)	2,000
Crude Herb powder mass (grams)	700
Solvent Volume (ml)	5,000
Extract Mass (grams)	75.3
Yield (%)	10.76

tract are described in Table 1. This showed that two kilograms of fresh lemon pepper formed an amount of 75.3 grams of concentrated lemon pepper extract. Due to this reason, the yield of lemon pepper extract was 10.76%.

Phytochemical screening of Lemon pepper methanol extract

After the physical characteristic evaluation of lemon pepper methanol extract was performed, this study continued the phytochemical screening of lemon pepper methanol extract, and the result of the phytochemical screening is described in Table 2.

Table 2 shows that lemon pepper methanol extract had some phytochemical compounds, including flavonoid, alkaloid, saponin and glycoside. Some previous studies by Saragih *et al*. (2019) and Saputra *et al*. (2018) have reported the phytochemical contents of lemon pepper extract, which was responsible for its pharmacologic properties. These pharmacologic properties included anti-inflammation, antioxidant, and antidiabetic activity, simultaneously postulated to reveal a kidney protection effect in diabetic conditions. Moreover, Saragih *et al*. (2019) reported that lemon pepper fruit had various phytochemicals like phenol, saponin, flavonoid, tannin, triterpenoid, and alkaloid. Both flavonoid and saponin have been widely investigated for antioxidant properties and antidiabetic activity by donating proton (hydrogen ion) into endogenous free radicals like Reactive Oxygen Species (ROS) from hyperglycaemic-related tissue damage. This study also demonstrated that lemon pepper extract, which had antioxidants, also neutralized streptozotocin-formed free radicals as reported by Saputra *et al*. (2018); Saragih and Arsita (2019).

Particle size analysis of nano-emulsion

This obtained lemon pepper methanol extract was further formulated into a nano-emulsion, and its particle size was analyzed to ensure the nano-size of the emulsion. This showed that the mean, median, mode, and ratio of mean to median from lemon pepper nano-emulsion were 0.2, 0.03, 0.01, and 5.00, respectively.

Interestingly, the present study used nano-emulsion pharmaceutical form of lemon pepper when some previous studies by Yanti *et al*. (2011), Saragih *et al*. (2018), Worotikan *et al*. (2017), and Chiuman *et al*. (2021) only looked for the pharmacologic properties of lemon pepper extract. Abdassah (2017) defined a nanoparticle as a drug nano-size-vehicle system that rapidly and directly delivers a drug substance into a specific area. Moreover, Mamillapalli *et al*. (2016) defined the nanoparticle size within 1 to 100 nanometres, increasing the drug bioavailability by increasing the contact surface area. This lemon pepper nano-emulsion obviously fulfilled the definition of nanoparticle, which was average of 0.2 µm. Hence, lemon pepper nano-emulsion can enhance its bioavailability.

Acute toxicity assay

Before this study continued to antidiabetic assay, the

Table 2. Phytochemicals screening of lemon pepper methanol extract

Phytochemicals	Methods	Result	References
Flavonoid	5% FeCl3	+	Suhartomi *et al.,* 2020
	Mg(s) + HCL(p)	-	Gulo *et al.* 2021
	NaOH 10%	-	Gulo *et al.* 2021
	H2SO4(p)	-	Gulo *et al.* 2021
Alkaloid	Bouchardart	+	Saragih *et al.* 2019
	Mayer	+	Saragih *et al.* 2019
	Wagner	+	Saragih *et al.* 2019
	Dragendorff	+	Saragih *et al.* 2019
Terpenoid and Steroid	Lieberman-Burchard	-	Saragih *et al.* 2019
	Salkowsky	-	Saragih *et al.* 2019
Tanin	1%FeCl3	-	Chiuman *et al.* 2023
Saponin	Distilled Water + 96% Alcohol	+	Chiuman *et al.* 2023
Glycoside	Mollish	+	Chiuman *et al.* 2023
Anthocyanin	2M HCl	-	Chiuman *et al.* 2023

Table 3. Acute toxicity assay of lemon pepper nano-emulsion

Dosage	Toxicity Signs			
	Locomotory activity changes	**Lethargy**	**Weakness or distress**	**Death**
2,000 mg/ kg BW	One minute seizure	One minute	Thirty seconds distress	Third minute
1,000 mg/ kg BW	Two minutes seizure	One minute	One minute distress	Fifth minute
500 mg/ kg BW	Four minutes seizures	Two minutes	One minute distress	Fifth minute
150 mg/ kg BW	Five minutes seizures	Two minutes	Thirty seconds distress	Sixth minute
50 mg/ kg BW	None	None	None	None

obtained lemon pepper nano-emulsion underwent acute toxicity assay as a preliminary test, and the acute toxicity assay is described in Table 3. This showed that lemon pepper nano-emulsion at a dose of 150-2,000 mg/ kg BW caused death within six minutes. The dose of 2,000 mg/ kg BW of lemon pepper nano-emulsion revealed the fastest death response, which was the third minute, followed by 500-100 mg/ kg BW, which was the fifth minute, and the slowest death response was found in the dose of 150 mg/ kg BW that was sixth minute. The death responses correlated to the duration of other toxicity signs; when the death response was fast, it was followed by the short duration of other toxicity signs. Thus, according to the acute toxicity assay, the safest dose of lemon pepper nano-emulsion was 50 mg/ kg BW. Due to this reason, the dose of nano-emulsion for lemon pepper nano-emulsion 1, 2, and 3 groups were 25 mg/ kg BW, 50 mg/ kg BW and 75 mg/ kg BW, respectively.

None of the previous studies used lemon pepper nano-emulsion; thus, this study performed an acute toxicity assay to determine its treatment and safe dose. The most safest dose of lemon pepper nano-emulsion was 50 mg/ kg BW. It was a lower dose than the extract form in some previous studies. One of these studies was Chiuman *et al*. (2022), who used lemon pepper methanol extract dose of 500-1,000 mg/ kg BW for kidney protection in diabetic nephropathy condition. Chiuman *et al*. (2022) used lower dose of lemon pepper ethanol extract for Non-Fatty Liver Disease in diabetic settings, that was 250-750 mg/ kg BW. Other study by Worotikan *et al*. (2017) also reported a fewer dose for kidney protection effect in diabetic settings, which was 150 to 300 mg/ kg BW. Based on previous studies by Worotikan *et al*. (2017) and Chiuman *et al*. (2022), compared to the results of the present study, a higher dose is required for the conventional pharmaceutical form, such as oral suspension, compared to the nano-sized emulsion form of lemon pepper extract. The nano-emulsion may improve drug delivery into the target organ; thus, it decreased the required dose for a similar pharmacologic property in common pharmaceutical form (oral suspension) and it also became obvious why all rat that received lemon pepper nano-emulsion higher than 50 mg/ kg BW suffered from acute toxicity signs (Mamillapalli *et al*., 2016).

Antidiabetic and kidney protection assay

The analysis also investigated the effects of lemon pepper nano-emulsion on antidiabetics and kidney protection. Initial data, initial body weight, were used to determine the received dose of lemon pepper nano-emulsion, and these data are mentioned in Table 4. This showed that all groups did not show any significant difference in initial body weight among all groups, as seen from P-Value > 0.05 (P-Value: 0.191). The highest tendency of initial body weight found in lemon pepper nano-emulsion 2 was 166 grams and the lowest was in the control group, 155 grams. After that, these groups received a certain amount of lemon pepper nano-emulsion based on their body weight. The last body weights of all groups was also measured at the

Table 4. Initial body weight of all groups

Group	Body Weight, *grams*	
	Intial	**Last**
Normal (Normal without treatment)	158 (156-261)	144 (101-236)
Control (0.5% SCMC suspension)	155 (152-183)	134 (114-174)
Standard (100 mg/kg BW of Metformin)	161 (152-177)	140 (138-160)
Lemon Pepper Nano-Emulsion 1 (25 mg/ kgBW)	162 (158-167)	138 (95-152)
Lemon Pepper Nano-Emulsion 2 (50 mg/ kgBW)	166 (164-167)	130 (75-156)
Lemon Pepper Nano-Emulsion 3 (75 mg/ kgBW)	162 (160-166)	108 (95-152)
P-Value	0.191	0.267

Data were expressed as Median (Min-Max); P-Value was obtained from Non-Parametric Statistic (Kruskal-Wallis

end of the study and are described in Table 4. The end body weight showed a similar result to intial body weight, which showed no significant body weight difference among all groups; it can be seen from P-Value > 0.05 (P-Value: 0.267). However, all groups concurrently showed to decrease the body weight. Thus, it indicated that lemon pepper nano-emulsion did not affect these rats' body weight.

Table 5 shows that no significant differences in initial blood glucose levels were observed among all treatment groups. It can be seen from P-Value > 0.05 (P-Value: 0.843). It indicated that all initial blood glucose levels tended to homogenous. After diabetic induction, all groups except normal groups underwent blood glucose increment, leading to significant blood glucose level differences (P-Value < 0.05). Moreover, blood glucose levels from standard and lemon pepper nano-emulsion groups gradually decreased after five, ten, and fifteen days of treatment. Oppositely, the blood glucose levels of control groups gradually increased until fifteen days of treatment. Last, the normal group acted as normal rats without any treatment or diabetic condition and this group did not show much blood glucose change.

Finally, all rats were sacrificed to obtain the blood via intracardiac puncture and it was used for kidney function test. The analysis of BUN and creatinine levels in all groups was mentioned in Table 6. It shows that lemon pepper nano-emulsion significantly affected BUN level, as seen from P-Value < 0.05 (P-Value: 0.01). The highest BUN level was found in the control group, followed by lemon pepper nano-emulsion 1, standard, lemon pepper nano-emulsion 2, and the lowest in lemon pepper nano-emulsion 3. However, lemon pepper nano-emulsion did not significantly affect creatinine level, as seen from P-Value > 0.05 (P-Value: 0.233).

Streptozotocin is a diabetogenic agent that has cytotoxic glucose analogue properties. It can affect both bacterial and human cells. Streptozotocin, which has nitrosourea moiety with a methyl group, enter the cell via the GLUT 2 transporter to fragment DNA sequences within 72 hours. DNA fragmentation begins with DNA methylation, followed by formation of nitric oxide and some free radicals like hydrogen peroxide. This process occurs within the pancreaticβ-cell, which leads to pancreatic dysfunction and hyperglycaemic condition.

Table 5. Blood glucose level of all groups

Group	Blood Glucose Level, mg/ dl				
	Before Induction*	**After Induction***	**5th day****	**10th day***	**15th day***
Normal	99 (85-101)	99 (85-101)	103.60 ± 8.5	111 (99-128)	76 (69-92)
Control	93 (88-105)	360 (278-456)	359.60 ± 79.98	369 (204-400)	340 (253-391)
Standard	99 (97-102)	176 (131-290)	191.00 ± 63.69	100 (86-201)	73 (70-83)
Lemon Pepper Nano-Emulsion 1	98 (87-105)	295 (202-491)	181.60 ±78.57	71 (63-86)	89 (83-99)
Lemon Pepper Nano-Emulsion 2	99 (91-101)	348 (240-482)	128.20 ± 41.68	78 (71-99)	96 (43-197)
Lemon Pepper Nano-Emulsion 3	97 (88-102)	297 (277-412)	157.60 ± 52.06	85 (51-90)	227 (138-350)
P-Value	0.843	0.003	< 0.05	< 0.05	0.001

*Blood Glucose level was expressed as Median (Min-Max) and P-Value was obtained from Non-Parametric Statistic (Kruskal-Wallis); **Blood Glucose level was expressed as Mean ± SD and P-Value was obtained from Parametric Statistic (One Way Anova)

Table 6. Blood urea nitrogen **(**BUN) and creatinine levels of all groups

Group	Kidney Function Test, mg/ dl	
	BUN	**Creatinine**
Normal	38 (34-46)	8 (2-44)
Control	59 (55-66)	17 (2-27)
Standard	44 (41-51)	8 (1-25)
Lemon Pepper Nano-Emulsion 1	49 (46-54)	27 (1-34)
Lemon Pepper Nano-Emulsion 2	40 (34-48)	27(10-31)
Lemon Pepper Nano-Emulsion 3	38 (36-38)	12 (1-29)
P-Value	0.001	0.233

Both data were expressed as Median (Min-Max) and P-Value was obtained from Non-Parametric Statistic (Kruskal-Wallis)

(Alotaibi *et al*., 2019; Eleazu *et al*., 2013)

The present study clearly demonstrated the antidiabetic and kidney protection activity of lemon pepper nano-emulsion, which was in line with previous studies by Chiuman *et al*. (2021, 2022) and Worotikan *et al*. (2017). These previous studies reported that 1,000 mg/ kg BW of lemon pepper ethanol extract significantly decreased the BUN and creatinine levels in diabetic nephropathy rats. Another study by Chiuman *et al*. (2021) also reported that lemon pepper methanol extract at a dose of 750 mg/ kg BW improves liver function in non-fatty liver diseases induced by streptozotocin. As discussed above, this study used lower doses of lemon pepper nano-emulsion due to its pharmaceutical form. These pharmacologic properties come from its phytochemicals that decrease blood glucose levels and prevent further hyperglycaemic-induced tissue damage. Alpha glucoside enzyme inhibition is a possible mechanism of action for lemon pepper's antidiabetic activity, preventing complex carbohydrate degradation and decreasing glucose absorption in gastrointestinal tract.

On the other hand, lemon pepper has also been reported to neutralize the oxidative stress process from hyperglycaemic-induced tissue damage and improve its sequel inflammation. Lemon pepper extract has been reported to have various phytochemicals. One of them was 2-methoxy4-vinilfenol, which has antioxidant effect to neutralize the oxidative stress process from hyperglycaemic-induced tissue damage. Other studies also demonstrated that lemon pepper extract inhibited the expression of some inflammation markers in LPS-induced macrophages model and led to decreased inflammation severity (Winarti *et al*., 2018; Yanti *et al*., 2011)

Conclusion

In conclusion, lemon pepper nano-emulsion effectively reduced blood glucose levels and mitigated kidney tissue damage in Streptozotocin-induced diabetic male Wistar rats, protecting against hyperglycemia-induced kidney injury. Each dose of lemon pepper extract formulated as nano-emulsions significantly lowered blood glucose levels, reaching levels close to normal by the tenth day. Specifically, the nano-emulsions reduced blood glucose by 71 mg/dL on the 10th day and 89 mg/ dL on the 15th day. Additionally, a dose of at least 50 mg/kg BW of lemon pepper nano-emulsion demonstrated kidney-protective effects, as evidenced by Blood Urea Nitrogen (BUN) levels approaching normal values (40 mg/dL), with the highest dose reaching 38 mg/dL. These effects showed a dose-dependent trend, where higher doses resulted in more favourable outcomes, likely due to the novel nano-formulation of the emulsion.

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Conflict of interest

The authors declare that they have no conflict of interest.

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