

Anti-Diabetic Potential of *Murraya Koenigii* (L.) and its Antioxidant Capacity in Nicotinamide-Streptozotocin Induced Diabetic Rats

Authors

Fauzul Husna^{1, 2}, Franciscus D. Suyatna³, Wawaimuli Arozal³, Erni H. Poerwaningsih⁴

Affiliations

- 1 Doctoral Programme in Biomedical Sciences Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
- 2 Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Aceh, Indonesia
- 3 Department of Pharmacology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
- 4 Department of Pharmacy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Key words

Diabetes, *Murraya koenigii*, oxidative stress, streptozotocin-nicotinamide

received 25.08.2017

accepted 09.10.2017

Bibliography

DOI <https://doi.org/10.1055/a-0620-8210>

Published online: 2018

Drug Res

© Georg Thieme Verlag KG Stuttgart · New York

ISSN 2194-9379

Correspondence

Franciscus D. Suyatna

Department of Pharmacology Faculty of Medicine

Universitas Indonesia

10430 Salemba Raya 4 Jakarta

Indonesia

Tel.: +62/818/409 779, Fax.: +62/21/3920 947

franciscus.suyatna@ui.ac.id; fransdsuyatna@yahoo.com

ABSTRACT

Aim and Objective The present study aims to investigate whether the antihyperglycemic effect of *Murraya koenigii* is mediated by antioxidant properties and insulin mimetic effect.

Methods Thirty Sprague-Dawley rats were induced hyperglycemia by streptozotocin and nicotinamide (STZ-NA). The STZ-NA diabetic rats were treated with an ethanolic extract of *Murraya koenigii* 200 mg/kg b.w and 400 mg/kg b.w. One group was treated with glibenclamide (1 mg/kg b.w). After the administration of *Murraya koenigii* extract and glibenclamide for four weeks, the rats were sacrificed. Blood and organ samples were collected under a fasting condition. The body weight and blood glucose levels were measured. Hepatic enzymes were determined using a commercial kit, protein levels were estimated by Bradford's method, and plasma insulin was assayed by an ELISA kit. Malondialdehyde (MDA) and reduced glutathione (GSH) levels were estimated by the TBA-Wills method and Ellman's method, respectively.

Results Ethanolic extract of *Murraya koenigii* showed a significant reduction in blood glucose level at both doses, 200 and 400 mg/kg b.w. In addition, *Murraya koenigii* exhibited a profound antioxidant effect with decreased MDA level and increased GSH level, particularly at the 200 mg/kg b.w. and significantly decreased the HOMA-IR index.

Conclusions The present study reveals that *Murraya koenigii* possesses antidiabetic activity and antioxidant effects on STZ-NA induced diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a complex and increasingly important medical problem in both developing and developed countries. It poses a serious healthcare issue worldwide. The global prevalence of DM is predicted to grow from 415 million or 8.8% of world's population in 2015 to 642 million by 2040 [1]. World Health Organization (WHO) estimated that Indonesia would be in rank fourth in the world for the most DM cases with estimated increases from 8.4 million in 2010 to 212.3 million by 2030 [2].

DM is distinguished by dysfunction of pancreatic β -cells along with decreased insulin secretion triggering chronic hyperglycemia.

Insulin deficiency with disruption in metabolism promotes various diabetic complications [3]. Free radicals play a part in the pathogenesis and complications of diabetes. Chronic and persistent hyperglycemia leads to oxidative stress via glucose autooxidation pathway, production of advanced glycation products, and polyol pathway. In the diabetic state, the endogenous defense mechanisms in scavenging free radicals have been impaired, and the failure of scavenging reactive oxygen species enhance oxidative damage in the tissue [4, 5].

Currently, available therapies for DM include diet modification, exercise, various oral antidiabetic agents, and insulin. However, long-term treatment using antidiabetic drugs contributes toward

side effects such as hypoglycemia, anorexia, and weight gaining. As a consequence, the use of the hypoglycemic drugs seems to be limited. In a clinical setting, the management of DM without any adverse effects is still a challenge and requires a great deal of study to find potentially useful natural, plant-derived agent [6]. Since antioxidants may have beneficial effects for improving various diseases, including DM, the plants which have antioxidant properties may have promising uses in therapy as adjunctive agents for diabetic condition and the plants with antidiabetic properties capable for the development of antihyperglycemia drugs [7, 8].

Murraya koenigii (L.) Spreng, also known as curry leaves, belongs to the family Rutaceae, which is widely distributed in the tropical and sub-tropical continent of South-East Asia and India. In Indonesia, *M. koenigii* is regularly used as spice and flavor enhancer in food processing. In the Ayurvedic system, *M. koenigii* is traditionally used to treat headaches, vomiting, influenza, insect bites, itching, rheumatism, dysentery, and diarrhea [9]. Some of these traditional uses have been verified by scientific studies, including its effects as a hypolipidemic [10], anti-tumor [11], antioxidant [12], and nephroprotective agent [13]. Many studies [14, 15] show that *M. koenigii* decreases blood glucose levels in animal models. However, only a few studies have confirmed that the phytochemical content and the mechanism of action in curry leaves improves the diabetic state. Thus, the present study was undertaken to investigate the phytochemical content as well as the antihyperglycemic and antioxidant activities of *M. koenigii* extract (MKE) in normal and DM rats.

Materials and Methods

Chemicals and animal

Streptozotocin (STZ), Bradford reagent, and tetramethoxypropane were purchased from Sigma, St. Louis, MO. Trichloroacetic acid, thiobarbituric acid, and nicotinamide were purchased from Merck, Germany. The bovine serum albumin, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and GSH were purchased from Sigma-Aldrich, USA. Glibenclamide was a gift from PT Kalbe Farma, Bandung, Indonesia. All other chemicals and reagents used in the study were analytical grade.

Male Sprague-Dawley weighing 170–250 g were purchased from the Animal Centre of The Board Health Research and Development Indonesia (Jakarta, Indonesia). All rats were housed in standard laboratory conditions during the experiment (temperature $23 \pm 2^\circ\text{C}$, 35–60% humidity with 12 h light-dark cycle). Rats were fasted overnight before the experiment. The animal experiments were carried out after the approval of the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (letter number 643/UN2.F1/ETIK/2015) and the studies conducted in appliance with the standard guidelines according to the NIH Guide for Care and Use of Laboratory Animals.

Preparation of extract

Fresh, green, mature curry leaves were obtained locally from Aceh, Indonesia in July 2015. The identity of the plant was confirmed by a taxonomist from the Indonesian Institute of Sciences, Bogor, In-

donesia. The voucher specimen of the plant was kept for future reference. One kilogram of curry leaves was dried at ambient temperature for 12–18 days. After drying completely, the leaves were ground to a coarse powder using an electric grinder.

The dry, powdered curry leaves were macerated in 96% ethanol with intermittent shaking. The alcoholic extracts were then filtered through a Whatman No. 1 and concentrated to dry mass under reduced pressure on a rotary evaporator at 50°C and stored at 4°C for future use.

Determination of % yield and phytochemical analysis

The phytochemical analysis were carried out for qualitative determination and a GC-MS analyzer was used to conduct a semi-quantitative analysis of the phytoconstituents in MKE and the percentage yield of MKE was estimated [16].

Acute toxicity study

The acute oral toxicity study was performed according to OECD guidelines for chemical testing [17]. The rats were observed individually for physical signs of toxicity such as writhing, gasping, palpitation and decreased respiratory rate or mortality constantly during the first 30 min, periodically during the first 24 h and daily thereafter for 14 days.

Induction of DM

Diabetes was induced by injection of STZ intraperitoneally (55 mg/kg) dissolved in 0.1 M cold citrate buffer (pH 4.5) 15 min after the intraperitoneal administration of nicotinamide at 120 mg/kg. After three days, the level of fasting plasma glucose (FPG) was measured from the tail vein using an Accu-Chek Performa glucometer (Roche Diagnostics Ltd. Co.Germany). The rats with $\text{FPG} \geq 250 \text{ mg/dL}$ were considered diabetic and included in the study.

Experimental design

The experiment was carried out on six groups of five rats each, all treated with test/standards for 30 days according to the following protocol:

Group 1: Normal rats treated with 0.8% CMC-NA solution (CON), Group 2: Normal rats treated with 400 mg/kg MKE (CON + MKE), Group 3: Diabetic rats treated with 0.8% CMC-NA solution (DM), Group 4: Diabetic rats treated with 200 mg/kg MKE (DM + L), Group 5: Diabetic rats treated with 400 mg/kg MKE (DM + H), Group 6: Diabetic rats treated with 1 mg/kg glibenclamide (DM + GLI).

MKE and glibenclamide suspensions, prepared by dispersion in 0.8% natrium-carboxymethyl cellulose (CMC-NA) solution, were administered orally via feeding canula once a day. The dose of MKE was selected based on previous report [12]. During the experimental period, the body weight and FPG of all rats were monitored weekly. At the end of the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected in tubes containing EDTA and the plasma was separated for use in estimating insulin level using an ELISA kit supplied by DRG, Germany (EIA-2048). Aspartate transaminase (AST) and alanine transaminase (ALT) levels were acquired by an enzymatic kit by Diasys® (International Holzheim, Germany). The liver tissue was excised, rinsed in ice-cold normal saline and homogenized in normal saline with a teflon homogenizer at 4°C .

The homogenate and plasma were used to estimate protein level by Bradford's method [18], malondialdehyde level was determined by the method TBA-Wills [19] and reduced glutathione was calculated by Ellman's method [20].

Statistical analysis

All data were presented as mean \pm SEM and statistical analysis was carried out by one-way ANOVA followed by LSD pos hoc using SPSS version 20. Values of $p < 0.05$ were considered statistically significant.

Results

Phytochemical analysis

Qualitative determination of phytoconstituents in MKE revealed the presence of flavonoids, steroids, and saponin. Additionally, a semi-quantitative analysis using GC-MS found that the major phytochemical components of MKE are 1.8- cineol, β -caryophyllen, hexadecen-1-ol, α -matrine, benzo[a]naphthacene, 2H-3,5A-epoxynaphth [2,1-B] oxepin, 12-epilycodoline, γ -sitosterol, noruns-12-ene and vitamin E (► Fig. 1). The % yield of the MKE was 18.98%.

Acute toxicity study

In the rats, oral administration of MKE in all doses did not produce any drug-induced physical signs of toxicity and no death was observed up to 14 days, indicating that MKE was non-toxic in rats up to an oral dose of 5000 mg/kg.

Effect of MKE on body weight

The rats' body weights were recorded in the fasting state every morning. The effect of the administration of MKE on body weight of the diabetic rats is presented in ► Table 1. After induction to DM, rats with DM exhibited symptoms of polydipsia, polyuria, and polyphagia. The body weights of the control rats increased by ± 57 g during the study period of 30 days. The body weights of diabetic rats decreased significantly compared to the CON group and the

administration MKE at the dose of 200 mg/kg b.w. ameliorated body weight in the diabetic group.

Effect of MKE on FPG and HOMA-IR index

Induction of DM in the experimental rats was confirmed by high levels of FPG and HOMA-IR. As shown in ► Table 2, there was a statistically significant increase in the FPG level and the index of HOMA-IR in the DM group was significantly large. In the diabetic rats, oral administration of MKE for 30 days significantly decreased FPG and MKE at the dose of 200 mg/kg b.w when compared to the does of 400 mg/kg b.w. However, the FPG and MKE levels were not significantly different in comparison with normal rats treated with MKE.

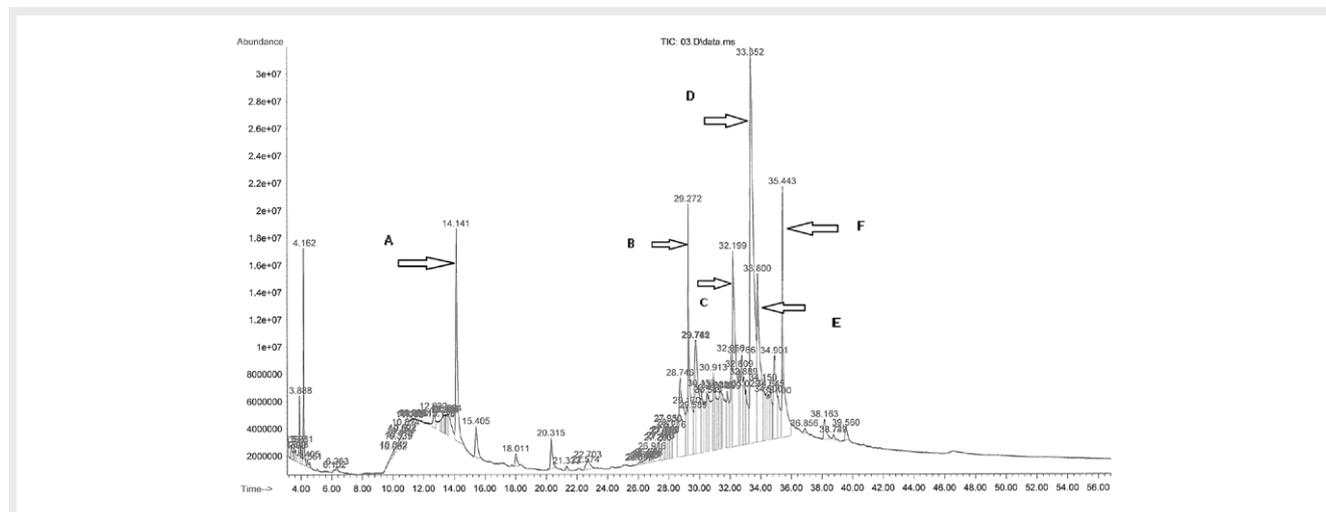
Effect of MKE on liver function markers and protein level

In our study, induction of DM increased ALT significantly when compared with the normal rats. Administration of MKE at doses of 200 and 400 mg/kg b.w. resulted in decreased ALT levels in the diabetic rats. The level of AST did not change significantly in any of the

► Table 1 Effect of MKE on body weight in STZ-NA induced diabetic rat

Groups	Body weight (g)	
	Initial	Final
CON	175.8 \pm 1.65	241.5 \pm 2.87
CON + MKE	184.0 \pm 2.50	206.0 \pm 5.60 ^a
DM	204.5 \pm 9.63	187.5 \pm 6.45 ^a
DM + L	207.5 \pm 4.35	218.0 \pm 12.03 ^b
DM + H	206.0 \pm 8.12	200.0 \pm 6.88 ^a
DM + GLI	238.5 \pm 3.77	200.0 \pm 2.16 ^a

Values are presented as mean \pm SEM (n = 5 for each group). ^a $p < 0.05$ vs. CON group; ^b $p < 0.05$ vs. DM group. CON control, DM Diabetic, DM + L diabetic with *Murraya koenigii* extract 200 mg/kg, DM + H diabetic with *Murraya koenigii* extract 400 mg/kg, GLI glibenclamide 1 mg/kg.



► Fig. 1 Phytochemical constituents of MKE by GC-MS analyzer. A. β -caryophyllene, B. hexadecen-1-ol, C. α -matrine, D. Benzo[a]naphthacene, E. 2H-3,5A-Epoxynaphth[2,1-B]oxepin and F. vitamin E.

► **Table 2** Effect of MKE on fasting blood glucose level in STZ-NA induced diabetic rat

Groups	Fasting blood glucose level (mg/dL)					HOMA-IR
	0 th Day	3 rd Day	7 th Day	14 th Day	30 th Day	
CON	92.2±7.89	100.8±8.29	105.4±10.50	102.8±3.96	107.4±14.29	2.49±0.21
CON+MKE	131.4±5.68 ^a	139.6±12.42	106.6±12.18	106.6±8.38	103.2±7.19	2.48±0.21
DM	102.6±10.71	368±39.22 ^a	473.6±174.71 ^a	387±84.41 ^a	407.8±115.07 ^a	18.79±3.01 ^a
DM+L	111.4±2.19	356±44.61 ^a	200.2±67.94 ^b	184.6±38.90 ^b	152.4±27.25 ^b	4.13±0.64 ^b
DM+H	105.2±7.19	334±59.10 ^a	191.4±62.63 ^b	197±115.46 ^b	212.8±123.11 ^b	7.53±2.08 ^{a,b}
DM+GLI	91.25±15.28	395.8±71.79 ^a	329.5±84.83 ^a	362.75±54.85 ^a	280.5±95.94 ^{a,b}	4.83±0.67 ^b

Values are presented as mean ± SEM (n = 5 for each group). ^ap < 0.05 vs. CON group; ^bp < 0.05 vs. DM group. CON control, DM Diabetic, DM + L Diabetic with *Murraya koenigii* extract 200 mg/kg, DM + H diabetic with *Murraya koenigii* extract 400 mg/kg, GLI glibenclamide 1 mg/kg.

► **Table 3** Effect of MKE on liver function enzyme and protein level in STZ-NA induced diabetic rat

Groups	Liver enzyme (U/L)		Protein Level
	ALT	AST	
CON	30.2±2	62.8±3	54.60±2.1
CON+MKE	32.9±3	70.3±4	58.90±1.1
DM	64.3±9 ^a	72.9±7	46.84±4.4
DM+L	33.2±4 ^b	61.5±3	57.12±4.9
DM+H	36.1±2 ^b	61.6±8	57.51±4.7
DM+GLI	81.8±9 ^a	79.6±11	71.0±8.0

Values are presented as mean ± SEM (n = 5 for each group). ^ap < 0.05 vs. CON group; ^bp < 0.05 vs. DM group. CON control, DM Diabetic, DM + L diabetic with *Murraya koenigii* extract 200 mg/kg, DM + H diabetic with *Murraya koenigii* extract 400 mg/kg, GLI glibenclamide 1 mg/kg.

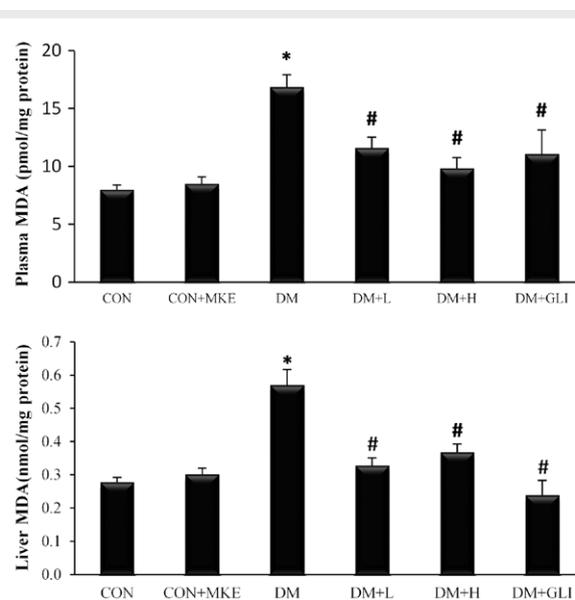
groups. Induction of DM decreased protein content compared to the normal rats but not significantly (► **Table 3**).

Effect of MKE on oxidative stress markers

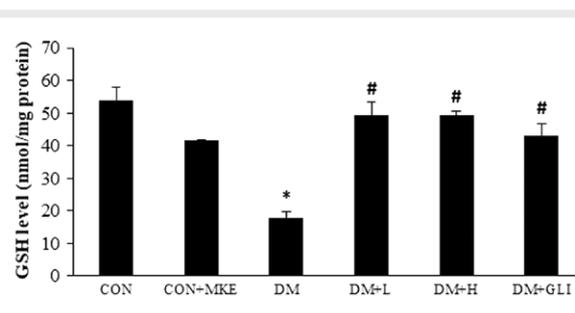
► **Fig. 2** and ► **Fig. 3** illustrate the effect of MKE on MDA and GSH levels in the control and diabetic groups. Upon the induction of diabetes, MDA level, both in plasma and liver tissue, increased and the GSH level decreased significantly compared with the control rats. The administration of MKE decreased MDA and increased GSH levels in the diabetic rats. Interestingly, MKE was superior to glibenclamide in restoring GSH in the diabetic rats. It is notable that MKE did not significantly change the antioxidant levels of the normal rats.

Discussion and Conclusion

The present study investigated the effect of MKE in a diabetic rat model to explore the antihyperglycemic effect and antioxidant capacity of curry leaves in order to establish pharmacological evidence and scientific support of the use of *Murraya koenigii* in the treatment of DM. We found that the antihyperglycemic action of MKE in STZ-NA-treated rats was mediated by its antioxidant properties. In addition, MKE could reduce insulin resistance on the diabetic rats. We used glibenclamide, which is a well known hypoglycemic drug commonly used as a standard antidiabetic drug in diabetes research to compare the efficacy of the variety of antihyperglycemic compounds [21, 22].



► **Fig. 2** Effect of MKE on level of MDA plasma (A) and liver tissue (B) on diabetic rats induced by STZ-NA. Values are presented as mean ± SEM (n = 5 for each group). * p < 0.05 vs. CON group. # p < 0.05 vs DM group. CON control, DM Diabetic, DM + L diabetic with *Murraya koenigii* extract 200 mg/kg, DM + H diabetic with *Murraya koenigii* extract 400 mg/kg, GLI glibenclamide 1 mg/kg. MDA malondialdehyde.



► **Fig. 3** Effect of MKE on GSH liver tissues level on diabetic rats induced by STZ-NA. Values are presented as mean ± SEM (n = 5 for each group). * p < 0.05 vs. CON group. # p < 0.05 vs DM group. CON control, DM Diabetic, DM + L diabetic with *Murraya koenigii* extract 200 mg/kg, DM + H diabetic with *Murraya koenigii* extract 400 mg/kg, GLI glibenclamide 1 mg/kg.

In line with this study, the plants with antihyperglycemic effects are now globally widely used as potential sources for drug discovery and development. Indonesia with the second highest biological diversity in the world is a potential country to develop this sort of study. Medicinal plants that contain biological substances including flavonoids, alkaloids, terpenoids, glycosides, etc. are often associated with antihyperglycemic effects. Thus, the formulation of herbal medicine should be studied intensively [23].

Murraya koenigii leaves contain various biological substances such as alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolics [24]. Our preliminary phytochemical screening of MKE showed the presence of flavonoids, steroids and saponin. Furthermore, we detected the presence of 1.8-cineol, β -caryophyllen, hexadecen-1-ol, α -matrine, benzo[a]naphthacene, 2 H-3,5 A-epoxynaphth[2,1-B]oxepin, 12-epilycodoline, γ -sitosterol, noruns-12-ene and vitamin E using GC-MS analyzer. Other studies showed that the major metabolite of *Murraya koenigii* were β -caryophyllene and α -humulene, carbazole alkaloids, β -sitosterol and furocoumarins [25, 26]. The variation in phytochemicals derives from specific environmental conditions. Differences in environmental conditions like UV radiation, rainfall, temperature, moisture, etc. generate variation of phytochemical content in the plants. Furthermore, the defense mechanism of plants in response to environmental stresses could raise metabolite production [27].

Diabetes mellitus is related to structural and functional alterations in the β -cells of the pancreas and a reduction in insulin secretion. The increased blood glucose and other biochemical abnormalities are caused by insulin deficiency and/or insulin resistance in target organs [3]. Various animal models which mimic diabetes in humans have been used in studying antihyperglycemic agents [26]. Specifically, to develop type 2 DM, many animal models have been developed, and are classified into spontaneously induced model and experimentally induced model. Using Zucker fa/fa rat is resemble for spontaneous model, but this model need strict controlled environment [28]. Considering our laboratory facilities, we used experimentally induced model. STZ, the derivative of nitrosourea, has been extensively used to induce diabetes in the animal model. STZ enhanced the production of free radicals and inhibited free radical scavenger systems that can induce oxidative stress and damage pancreatic β -cells. The administration of STZ selectively destroys pancreatic β -cells leading to a deficiency in insulin secretion, which leads to hyperglycemia. The injection of NA prior to the administration of STZ to the rats to induce the diabetic condition with reduced insulin and stable metabolic change, which effectively mimics human DM [29, 30]. In our study, NA was administered at 120 mg/kg and STZ at 55 mg/kg, significantly increasing FBG. This result is consistent with a number of studies that indicate that STZ-NA produces a model of hyperglycemia [31, 32].

Hyperglycemia is marked by signs of polydipsia, polyuria, and weight loss. The drop in body weight is linked to proteolysis in the skeletal muscle and the degradation of fats caused by unavailability of energy source from carbohydrate [33]. In our study, weight loss was exhibited by the diabetic rats, indicating a progressive proteolysis caused by the derangement of the metabolism of carbohydrates. The administration of MKE at the dose of 200 mg/kg b.w significantly improved the regulation of body weight, reflecting a

better control of glycemic status that possibly prevented muscle wasting.

HOMA-IR is a parameter which is widely used in determining the gradation of insulin resistance. This index is calculated from fasting plasma glucose and insulin levels. We found HOMA-IR was increased in diabetic rats and that the administration of MKE restored the HOMA-IR index to near normal levels. Interestingly, diabetic rats treated with MKE at a dose of 200 mg/kg b.w exhibited greater improvement in insulin resistance than those treated with a MKE dose of 400 mg/kg b.w.

Hyperglycemia is one of the sources of free radical generation via glucose auto-oxidation and the polyol pathway [34]. Oxidation of the phospholipid membrane excessively promotes ROS production, which inhibits the tissue's utilization of glucose [5]. The liver is a principal organ involved in the detoxification process and the utilization of glucose. Liver intensively mobilizes and reserves absorbed glucose to maintain the blood glucose level in the normal state. Membrane breakdown impairs physicochemicals of tissue and causes the leakage of cytoplasmic enzymes. The increased level of cytoplasmic enzymes in the liver indicates functional disintegrity of the membrane structure of the liver [35]. In the present work, ALT level was increased in the diabetic groups and the administration MKE restored ALT levels to near normal. These findings reveal that MKE restores hepatic function in the diabetic condition. In the glibenclamide group, we are still looking for an explanation of the increased liver enzyme activities. At this point, we could explain that it is possible that the control of FBG that has not reached the normal level in the glibenclamide group probably causes hyperglycemia that increased glucose autooxidation and further burdens the organs involved in glucose metabolism including the liver. This causes an increase in the activity of the liver that potentially damages the liver, further study needed to clarify this issue.

Persistent hyperglycemia impairs the prooxidant/antioxidant balance, reduces the level of antioxidants, and advances the production of reactive oxygen species. The human body has a defense system which minimizes oxidative stress by the involvement of enzymatic and non-enzymatic antioxidative systems [36]. The increased level of MDA could mediate the generation of free radicals and reduce the activities of antioxidants enzymes. GSH is a second-line intracellular antioxidant that inhibits ROS generation and lipid peroxidation. GSH depletion impairs thiol redox state and exacerbates oxidative stress. Our study found that the oral administration of MKE reduced plasma and liver MDA levels and restored the depleted GSH to near normal in the diabetic rats. The results support the suggestion of antioxidant activity of MKE in the diabetic condition.

To conclude, the present study estimated oxidative stress markers in STZ-NA induced diabetic rats and the effect of ethanolic *Murraya koenigii* extract in the amelioration of the diabetic state. The phytochemical analysis conducted revealed the presence of biologically active substances such as β -caryophyllen, α -matrine, benzo[a]naphthacene, 2 H-3,5 A-epoxynaphth[2,1-B]oxepin, 12-epilycodoline, and vitamin E, which could be mediated for their antihyperglycemic effects. The antihyperglycemic effect of MKE could result from its antioxidant activities and decreased insulin resistance. Our study confirmed that MKE could have an anti-diabetic effect at least in part via its antioxidant properties.

Acknowledgement

The authors thank the Directorate Research and Community Services, Universitas Indonesia, Indonesia for providing financial support.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] IDF. IDF Diabetes Atlas. International Diabetes Federation 2015; 1–163
- [2] Soewondo P, Ferrario A, Tahapary DL. Challenges in diabetes management in Indonesia: A literature review. *Global Health* 2013; 9: 63–80
- [3] Diabetes DOF. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; 33: (Suppl 1):
- [4] Pazdro R, Burgess JR. The role of vitamin E and oxidative stress in diabetes complications. *Mech Ageing Dev*. 2010; 131: 276–286
- [5] Brownlee M. A radical explanation for glucose-induced beta cell dysfunction. *J Clin Invest*. 2003; 112: 1788–1790
- [6] Pethe M, Yelwatkar S, Manchalwar S et al. Evaluation of Biological Effects of Hydroalcoholic Extract of Hibiscus Rosa Sinensis Flowers on Alloxan Induced Diabetes in Rats. *Drug Res (Stuttg)* 2017; 67: 485–492
- [7] Patel DK, Prasad SK, Kumar R et al. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012; 2: 320–330
- [8] Arulselvan P, Ghofar HAA, Karthivashan G et al. Antidiabetic therapeutics from natural source: A systematic review. *Biomed Prev Nutr* 2014; 4: 607–617
- [9] Chakraborty DP, Barman BK, Bose PK. On the constitution of murrayanine, a carbazole derivative isolated from *Murraya koenigii* Spreng. *Tetrahedron* 1965; 21: 681–685
- [10] Birari R, Javia V, Bhutani KK. Antiobesity and lipid lowering effects of *Murraya koenigii* (L.) Spreng leaves extracts and mahanimbine on high fat diet induced obese rats. *Fitoterapia* 2010; 81: 1129–1133
- [11] Khan BA, Abraham A, Leelamma S. Role of *Murraya koenigii* (curry leaf) and *Brassica juncea* (Mustard) in lipid peroxidation. *Indian J Physiol Pharmacol*. 1996; 40: 155–158
- [12] Ningappa MB, Dinesha R, Srinivas L. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chem*. 2008; 106: 720–728
- [13] Yankuzo H, Ahmed QU, Santosa RI et al. Beneficial effect of the leaves of *Murraya koenigii* (Linn.) Spreng (Rutaceae) on diabetes-induced renal damage in vivo. *J Ethnopharmacol*. 2011; 135: 88–94
- [14] Yadav S, Vats V, Dhunoo Y et al. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *J Ethnopharmacol*. 2002; 82: 111–116
- [15] Kesari AN, Kesari S, Singh SK et al. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. *J Ethnopharmacol*. 2007; 112: 305–311
- [16] Harborne JB. *Phytochemical Methods A Guide To Modern Techniques Of Plant Analysis*. Third Edition Chapman Hall; 1998 58:
- [17] OECD. *Guidance Document on Acute Oral Toxicity Testing*. 2001; 1–24
- [18] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72: 248–254
- [19] Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem*. 1978; 86: 271–278
- [20] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959; 82: 70–77
- [21] Aboonabi A, Rahmat A, Othman F. Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Toxicol Reports* 2014; 1: 915–922
- [22] Shukla K, Dikshit P, Tyagi MK et al. Ameliorative effect of Withania coagulans on dyslipidemia and oxidative stress in nicotinamide-streptozotocin induced diabetes mellitus. *Food Chem Toxicol*. 2012; 50: 3595–3599
- [23] Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*. 2002; 81: 81–100
- [24] Singh AP, Wilson T, Luthria D et al. LC-MS-MS characterisation of curry leaf flavonols and antioxidant activity. *Food Chem*. 2011; 127: 80–85
- [25] Nagappan T, Ramasamy P, Wahid MEA et al. Biological activity of carbazole alkaloids and essential oil of *murraya koenigii* against antibiotic resistant microbes and cancer cell lines. *Molecules* 2011; 16: 9651–9664
- [26] Ma Q, Tian J, Yang J et al. Bioactive carbazole alkaloids from *Murraya koenigii* (L.) Spreng. *Fitoterapia* 2013; 87: 1–6
- [27] Chaves MM, Pereira JS, Maroco J et al. How plants cope with water stress in the field. Photosynthesis and growth. *Ann Bot*. 2002; 89 (SPEC. ISS.): 907–916
- [28] Fröde TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. *J Ethnopharmacol*. 2008; 115: 173–183
- [29] Masiello P, Broca C, Gross R et al. Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* 1998; 47: 224–229
- [30] Akahane K, Inoue T, Yokoyama A et al. Efficacy of Mitiglinide Combined with Dapagliflozin in Streptozotocin-nicotinamide-induced Type 2 Diabetic Rats and in Zucker Fatty Rats. *Drug Res (Stuttg)* 2014; 65: 416–421
- [31] Arya A, Cheah SC, Looi CY et al. The methanolic fraction of *Centrathrum anthelminticum* seed downregulates pro-inflammatory cytokines, oxidative stress, and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats. *Food Chem Toxicol*. 2012; 50: 4209–4220
- [32] Tahara A, Matsuyama-Yokono A, Shibasaki M. Effects of antidiabetic drugs in high-fat diet and streptozotocin- nicotinamide-induced type 2 diabetic mice. *Eur J Pharmacol*. 2011; 655: 108–116
- [33] Kasetti RB, Rajasekhar MD, Kondeti VK et al. Antihyperglycemic and antihyperlipidemic activities of methanol: Water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food Chem Toxicol*. 2010; 48: 1078–1084
- [34] Yaribeygi H, Mohammadi MT, Sahebkar A. PPAR- α Agonist Improves Hyperglycemia-Induced Oxidative Stress in Pancreatic Cells by Potentiating Antioxidant Defense System. *Drug Res (Stuttg)* 2018
- [35] Palsamy P, Sivakumar S, Subramanian S. Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. *Chem Biol Interact*. 2010; 186: 200–210
- [36] Evans JL, Goldfine ID, Maddux BA et al. Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endocr Rev*. 2002; 23: 599–622