ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effect of Ethanol Extracts of Mustard Green (*Brassica rapa L.*) on Streptozotocin Induced Rats

Sri Wahjuni*, Ni Wayan Bogoriani

Department of Biochemistry, Universitas Udayana, Bali Indonesia.

*Corresponding Author: **Sri Wahjuni**

Abstract

Objective: This experimental study was conducted to evaluate the effect of the mustard green extract on blood glucose and the pancreas histopathological feature. Methods: Five groups of rats divided into the control group that received standard food (P0) and streptozotocin-induced group (P1, P2, P4, and P4). P1 (positive control group) was given glibenclamide; P2, P3, and P4 (treatment group) were given mustard green ethanol extract at a dose of 0.5; 2.0; and 5.0 mg/kg body weight/day respectively. The separation of ethanol extract of mustard green was carried out by LC-MS/MS. One way ANOVA and Post hoc test was conducted to evaluate the mean difference of the blood glucose. Results: Post hoc test showed significant result for P0 vs P3 ($p<0.001$), P0 vs P4 ($p=0.001$), P1 vs P2 ($p=0.048$), P2 vs P3 ($p=0.001$). Comparison between P1 and treatment group was found a similar effect on blood glucose between P1 vs P3 ($p=0.048$) and P1 vs P4 ($p=0.830$). Histological studies showed the administration of ethanol extract of mustard green showed a restorative effect. Conclusion: Administration of ethanol extract of mustard green decrease the level of blood glucose and might be a usable treatment for hyperglycemia.

Keywords: *Mustard green (Brassica rapa L.), Streptozotocin, Hyperglycemic effects.*

Introduction

Diabetes mellitus is a primary disorder of carbohydrate metabolism, which generally involves absolute or relative insulin deficiency and insulin resistance and ultimately leads to hyperglycemia. There has been an increasing demand for the use of natural products with antidiabetic activity. The undesirable side effects of synthetic drugs, easier consumption or availability and the fact that they are not suitable for use during pregnancy, were factors that lead strong desire to use hypoglycemic agents of plant origin [1-3].

Some herbs and plant products have been shown to have antihyperglycemic action [4-6]. Plants may act on blood glucose through different mechanisms. Some of them may have insulin-like substances [7, 8]; some may inhibit insulinase activity while others may increase beta cells in the pancreas by activating regeneration of these cells [9,10]. However, very few of the traditional treatments for diabetes have received scientific scrutiny.

This study aimed to investigate the effect of green mustard used in traditional medicine in the treatment of diabetes and its effect on the tissue of the pancreas.

Materials and Methods

Preparation of the Animals

Male Wistar weighing 180-200g collected from the Department of Biochemistry Animal House, Universitas Udayana, Indonesia. Thirty rats were cared on dry and clean plastic cages, with a cycle of light-dark for 12 hours at 25 $\pm$ 2°C and relative humidity at 45-55%. The animals were fed with pelletized commercial rat feed (Pfizer Livestock Co. Ltd) and water on demand. The Wistar rats divided into five groups and classified based on their initial blood glucose. Each group consist of six rats.

Sample Collection

Samples of green mustard (*Brassica rapa L.*) obtained from markets in Denpasar, Bali, Indonesia.

The authentication process of plant materials was conducted by Ir. Tuah Malen Bangun., M.Si, taxonomist of the Department of Botany Bedugul, Bali.

Preparation of Ethanol Extract of Green Mustard

Extraction: Powder of mustard green extracted using 96% ethanol by maceration process until immersed with the solvent. The clear filtrate obtained after repeatedly ±48 hours soaking process. Complete extraction confirmed by the LC-MS/MS machine with thin layer contained clear filtrate then rotary vacuum used to separate the ethanol extract from the solvent. A thick ethanol extract of mustard green produced from the evaporation process. Fractionation of the thick ethanol extract using ethyl acetate, n-hexane, and water produce the fractionated product. Dosage form from the evaporation of fractionated product used as the treatment in this study.

Animal Treatments

The five groups of rats were as follows: P0 or normal group (received standard food only); P1 or positive control group (streptozotocin induction and treated with glibenclamide); treatment groups or P2, P3, and P4 (streptozotocin induction and treated with mustard green ethanol extract). The dose of streptozotocin for hyperglycemia induction was 125 mg/kg body weight. Hyperglycemia was confirmed one week after streptozotocin injection. The rats were adapted with intra-peritoneal injection of streptozotocin until seven days, then treated with 0.5; 2.0; and 5.0 mg/kg body weight of mustard green by oral administration.

After one month, blood sample collection from cardiac puncture kept into a tube containing anticoagulant (fluoride/oxalate) then measured within 24 hours for the blood glucose.

Histopathological Evaluation

Pancreas tail parts removed on the last day experiment and preserved with 10% formaldehyde. Autotechnicon used to process the tissue. Slides with a mounted section with 5µ thick and stained with HE (hematoxylin & eosin) and evaluated morphologically by microscopic examination on 525 times magnification.

Statistical Analysis

All data from the research were analysed statistically using the bivariate and multivariate test method with the SPSS program (Statistical Product and Service Solution) and described as means ± SD (standard deviation). Post hoc test used to compare the mean difference between the group. Differences in mean values were considered significant at $p < 0.05$.

Results

Brassica Rapa L. Extract Effects on Blood Glucose Level

The Normality test was conducted to evaluate the data distribution. Measurement of normality test resulted with p>0.05 in each group for mean difference (Δmean) of blood glucose (Table 1). The blood glucose level in each group (n=6) were recorded from the initial day before treatment and one month after treatment. The mean blood glucose in each group presented in Table 2.

Table 1: Normality test

Groups	Pre-treatment	Post-treatment	Δmean of blood glucose
P0	0.212*	0.607*	0.830*
P1	0.136*	0.152*	0.573*
P2	0.212*	0.167*	0.514*
P3	0.001	0.698*	0.709*
P4	0.026	0.753*	0.607*

*Data normally distributed if p>0.05 (Shapiro-Wilk test)

Table 2: Glucose level among groups

Groups	Glucose level (mean ± SD) (mg/dL)				
	Pre-treatment	Post-treatment	p-value	Δmean	p-value
P0	108.83±0.75	109.00±1.79	0.822□	-0.167±1.72	<0.001*•
P1	321.50±3.45	301.33±9.20	0.003*□	20.17±8.84	
P2	257.83±0.75	248.00±0.89	<0.001*□	9.83±1.32	
P3	248.33±4.13	223.00±6.69	0.027*▪	25.33±9.22	
P4	105.50±5.05	89.00±2.44	0.027*▪	16.50±10.59	

*significant mean difference if p<0.05; □two-related sample conducted with Paired T-test; ▪two-related sample conducted with Wilcoxon test; •one way ANOVA test

ANOVA test was conducted to evaluate the blood glucose difference between the group. Mean difference (Δmean) of blood glucose level among groups were p<0.001 (Table 2). The post hoc test was conducted to evaluate the correlation between each group (Table 3).

Table 3: Post hoc test for difference of blood glucose level between groups

Tukey multiple comparison test	Mean difference	95% CI		p-value
		Lower bound	Upper bound	
P0 vs P1	20.33	10.07	30.59	<0.001*
P0 vs P2	10.00	-0.259	20.25	0.059
P0 vs P3	25.50	15.24	35.75	<0.001*
P0 vs P4	16.67	6.40	26.92	0.001*
P1 vs P2	-10.33	-20.59	-0.07	0.048*
P1 vs P3	5.16	-5.09	15.42	0.585
P1 vs P4	-3.66	-3.67	-13.92	0.830
P2 vs P3	15.50	5.24	25.75	0.001*
P2 vs P4	6.67	-3.59	16.92	0.339
P3 vs P4	-8.83	-19.09	1.42	0.11

*significant mean difference if p<0.05

Histopathology Changes of Pancreas

Cellular integrity and architecture were intact in the P0 group (Figure 1). Pancreatic sections stained with hematoxylin and eosin (H & E) showed that streptozotocin caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Nuclear changes, karyolysis, the disappearance of the nucleus and in some places, the residue of destroyed cells were visible (Figure 2, 3, 4 and 5). Relative reduction of size and number of islets, especially around the central vessel and severe reduction of beta cells were seen (Figure 2 and 4). A higher dose of the extract has a greater restorative effect on the islet cells of diabetic rats than a lower dose of extract. There was no significant effect of the extract on the pancreas of normal rats.

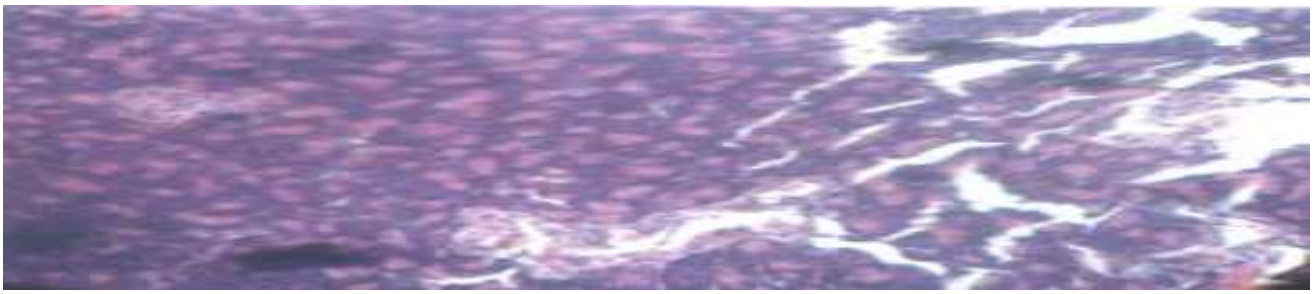

Figure 1: Pancreas of normal healthy rat (normal control/P0)

Figure 2: Pancreas of hyperglycemic control rat (given glibenclamide/P1

Figure 3: Pancreas of Hyperglycemic rat given with 0.5 mg/kg extract (P2)

Figure 4: Pancreas of hyperglycemic rat given 2.0 mg/kg extract (P3)

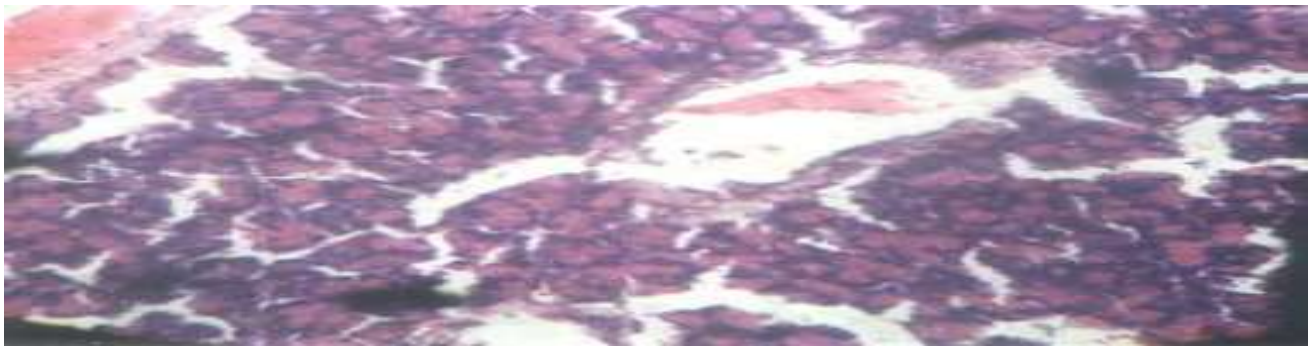

Figure 5: Pancreas of normal blood glucose rat treated with 5.0 mg/kg extract (P4)

Discussion

Before the treatment started, the blood glucose concentration of the P0 (108.83 mg/dl) P4 (105.5 mg/dl) are considered normoglycemic, while P1 (321.5 mg/dl), P2 (257.83 mg/dl), and P3 (248.33 mg/dl) are considered hyperglycemic [2, 11]. Significance effect for lowering blood glucose in the P1 considered as the control for the hyperglycemic group.

The significant decrease for blood glucose found in the P2, P3, and P4. In the P2 group, blood glucose decreased by 9.83 mg/dl ($p<0.001$). Blood glucose decreased for 25.33 mg/dl ($p=0.027$) in the P3. For the normoglycemic group (P4), blood glucose decreased for 16.50 mg/dl after one month ($p=0.027$).

Based on the multivariate analysis, the results showed that the hyperglycemia control group (P1) has a significant effect on the lowering blood glucose compared to the normal group (P0) (95% CI: 10.07 – 30.59; $p<0.001$). The effect of mustard green extract at dose 0.5 mg/kg body weight on lowering blood glucose based on the mean difference is lower significantly compared to the normal hyperglycemic group ($p=0.048$).

This compassion showed dose of mustard green at dose 0.5 mg/kg body weight to decrease the blood glucose is not similar compared to the glibenclamide, despite the dose of 0.5 mg/kg body weight decrease the blood glucose and the result is significant while comparing the pretreatment and post-treatment result in P2 ($p<0.001$).

The comparison of blood glucose means the difference between P1 and P3 showed similarly or not significantly different ($p=0.585$). The same result also found in the comparison between P1 and P4 ($p=0.830$). This result showed a similar effect between glibenclamide 125 mg/kg body weight and the dose of mustard green at dose 2.0 mg/kg body weight and 5.0 mg/kg body weight to decrease the blood glucose.

Comparison between P3 and P4 showed a not significant result ($p=0.11$). The dose given to P3 and P4 seems to have a similar effect on the lowering blood glucose. The mean difference for lowering blood glucose found in the group that received 2.0 mg/kg body weight of the mustard green extract. Although the blood-glucose-lowering effect is similar between P1 and P4, the lower mean difference in the P4 group might due to the physiological feedback to maintain the circulation blood glucose in the normal level and prevent the hypoglycemic state.

The findings may indicate the presence of some hypoglycemic agents in the ethanol mustard green, which have been concentrated in the extracts. The

hypoglycemic effects of plants may be due to the presence of an insulin-like substance in plants [7,8], stimulation of ß cells to produce more insulin [12,13], increasing glucose metabolism [14] or regenerative effect of plants on pancreatic tissue [10].

In this study, the pancreatic ß cells were destroyed with the induction of streptozotocin. Streptozotocin is one of the usual substances used for the induction of diabetes mellitus apart from alloxan [15-17]. Streptozotocin has a destructive effect on the beta cells of the pancreas [15]. The pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood.

In response to elevated blood glucose, insulin is secreted. Histopathological study of diabetic rats (P2) showed degeneration of pancreatic islet cells, which was due to streptozotocin used in this study. This condition probably gave rise to insulin deficiency. Insulin deficiency (or diabetes mellitus) causes an excessive elevation of blood glucose and underutilization, leading to hyperglycemia [18]. The histopathological study of the treated diabetic group (P1, P2, and P3) indicated increased volume density of islets and increased the percentage of beta cells in the diabetic rats that received the extracts, which may be a sign of regeneration. Signs of regeneration of ß cells, potentiation of insulin secretion from surviving ß cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts [3, 9,10,19-21].

Conclusion

Ethanol extract of mustard green showing hypoglycemic effect in hyperglycemia rats. Histopathological evaluation showing regeneration cell on the pancreas of the rats treated with mustard green. These findings support the use of mustard green for the treatment in hyperglycemia condition and might be useful for diabetes treatment.

References

1. Berger W. Incidence of severe side effects during therapy with sulphonylureas and biguanides. Hormones Metabolic Res. 1985; 17: 111-115.
2. Jeloder G, Mohsen M, Shahram S. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. African J. Traditional, Complementary and Alternative Medicines (Afr. J. Trad. CAM). 2007; 4(3): 299-305.
3. Yadev JP, Saini S, Kalia AN and Dangi AS. Hypoglycemic activity of ethanolic extract of Salvadora oleoides in normal and alloxan-induced diabetes rats. Indian J. Pharmacol. 2008; 40(1): 23-27
4. Elder C. Ayurveda for diabetes mellitus. A review of the biochemical literature. Altern. Ther. Health Med. 2004; 10: 44-50.
5. Srinvasan K. Plant foods in the management of diabetes mellitus: spices as beneficial antidiabetic food adjuncts. Int. J. Food Sci. Nutr. 2005; 56: 399-414.
6. Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S. Antihyperglycemic activity of aqueous extract of leaves of Cocculus hirsutus (L.) Diels in alloxan-induced diabetic mice. Indian J. Pharmacol. 2006; 38: 49- 53.
7. Rahmatullah M, Shefa TF, Hasan L, Hossain MT, Ahmed S, Al Manun A, Islam MR, Rahman S, Chowdhury MH. A Study on Antinociceptive and Anti-hyperglycemic Activity of Methanol Extract of Brassica juncea (L.) Czern. Leaves in Mice. Advances in Natural and Applied Sciences. 2010; 4(3): 221-225.
8. Wahjuni S, Laksmiwati AAIAM, Bogoriani IW. Administration of ethanol extract of mustard greens (Brassica rapa L) leaves increased Superoxide Dismutase levels in Hyperglycemic rat. Journal of Physics: Conference Series. 2019; 1341: 1-6
9. Abdel Monein A, El-Feki M and Salah E. Effect of Nigella sativa, fish oil and gliclazide on alloxan diabetic rats. Biochemical and Histopathological studies. J. Egy. Ger. Soci. Zool., 1997; 23: 237-265.
10. Shanmugasundaram ER, Gopith KI, Radha SK and Rajendram VM. Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given Gymnema sylvestere leaf extract. J. Ethnopharm. 1990; 30: 265-269.
11. Mayne PD. Clinical Chemistry in diagnosis and treatment. 6th ed. Edward Arnold (Publishers) Ltd., London. 1994: pp.206-219.
12. Chang MW, Johnson MA. Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. J. Nutr. 1980; 110: 931-936.
13. Khan A, Bryden NA, Polasky MN, Anderson

RA. Insulin-potentiating factor and chromium content of selected spices. Biol. Trace Elem. Res. 1990; 24: 183-188.

14. Broadhurst CL. Nutrition and non-insulin diabetes mellitus from an anthropological perspective. Alt. Med. Rev. 1997; 25: 378-399.
15. Ei-Soud NH, Khalil MY, Oraby FS, Farrag AR. Antidiabetic effects of Fenugreek alkaloid extract in streptozotocin-induced hypoglycemic rats. J. App. Sci., 2007; 3: 1073-1083.
16. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Allah Verdi A, Mofidian SMA, Lame Rad B. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry. 2007; 22(2): 60-64.
17. Nagarachi K, Ahmed S, Sabus A, Saheb SH. Effect of Streptozotocin on Glucose levels in Albino Wister Rats. Journal of Pharmaceutical Sciences and Research. 2015; 7(2): 67-69.
18. Standl E, Ballestshoffer B, Dahl B, Shagler H. Predictors of 10 years macrovascular and overall mortality in patients with NIDDM: the Munich practitioner project. Diabetologia. 2003; 39: 540-545.
19. Ayber MJ, Riera AN, Grau A, Sanchez SS. Hypoglycemic effect of the water extract of Smallanthus soncifolius (Yacon) leaves in normal and diabetic rats. J. Ethnopharmacol. 2001; 74: 125-132.
20. Suba V, Muragesan T, Bhaskara RR, Ghosh L, Pat M, Mandal SC, Saha BP. Antidiabetic potential of Barleria lupulina extract in rats. Fitoterapia. 2004; 75: 1- 4.
21. Wahjuni S, Gunawan IWG, Malindo, IYD. The effect of mustard greens (Brassica rapa l.) Ethanol extract on blood glucose and malondialdehyde levels on hyperglygemic wistar rats. Bali Medical Journal. 2009; 8(1): 35-40.

