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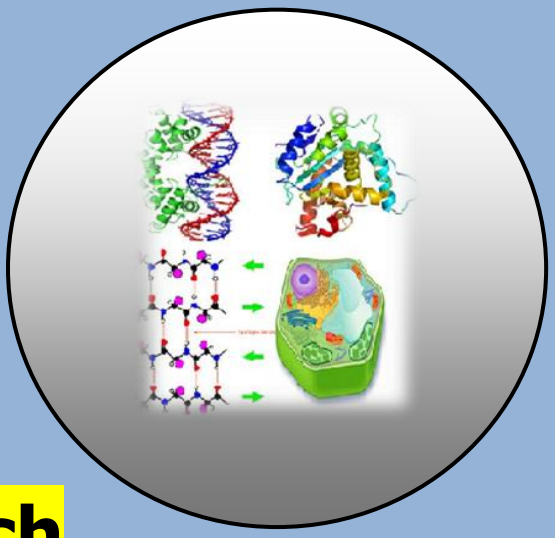
ISSN 0970-4973 Print
ISSN 2319-3077 Online/Electronic

Global Impact factor of Journal: 0.756
Scientific Journals Impact Factor: 3.285
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (1) 2015 Pages No. 299-312



Journal of Biological and Chemical Research

An International Journal of Life Sciences and Chemistry

Indexed Abstracted and Cited in about 25 different Scientific Databases around the World

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 1: 299-312, 2015
(An International Journal of Life Sciences and Chemistry)
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ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER

Received: 15/01/2015 Revised: 13/02/2015 Accepted: 28/02/2015

Effects of Pranajiwa Seed Extract Repair Damaged Pancreatic B-Cells through Decrease of Blood Glucose Levels, *Advanced Glycation end-Products* and Profile Histopathology in Hyperglycemic Rats

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ABSTRACT

Increased production of free radicals is one factor causing the onset of hyperglycemia. Chronic hyperglycemia increases the formation of reactive oxygen species (ROS), causing oxidative stress. This study aims to determine the effectiveness of seed extract in fixing the rate of β-cell pancreas damage in hyperglycemia rat wistar induced with alloxan.

The study applying a randomized pre-and posttest control group design with dose of pranajiwa seed extract. A total of 40 Wistar rats were used in this study. Rats were divided into four groups: one control group and three treatment groups with different doses of pranajiwa seed extract respectively 0.5 mg/kg bw, 2 mg/kg bw, and 5 mg/kg bw. All control treatment groups have alloxan induced at a dose of 125 mg/kg bw to obtain hyperglycemia.

The results showed that, intake of glibenclamide in positive control group blood glucose level to 110,76 mg/dL. For treatment group on the other hand, intake seed extract at P1 reduced blood glucose level to 138.59 mg /dL, at P2 to 128.39 mg/dL and at P3 to 103.69 mg/dL respectively. The mean levels of AGEs in the positive control group (glibenclamide) was 0.049 mol/L, the treatment group was 0.089 mol/L for P1, 0.067 mol/L for P2, and 0.038 mol/L for P3. The mean reduction levels of blood glucose and AGEs, were significantly different ($p<0.05$).

It can be concluded that the administration of pranajiwa seed extract at a dose of 5 mg/kg bw can reduced the rate of destruction of β-cell damage and structural changes of pancreatic β-cells tissue histopathology in rats wistar hyperglycemic patients can be improved.

Keywords: Pranajiwa, Blood glucose, AGEs, Histopathology and Hyperglycemia.

INTRODUCTION

Hyperglycemia is a condition where there is an increase in patients with fasting blood glucose levels above 110 mg/dl and 2-hour blood glucose pp (*post prandial*) above 140 mg/dl (PERKEMI, 2012). Hyperglycemia can increase the compound reactive oxygen species (ROS) through the process of enzymatic reactions are oxidation and phosphorylation (ox-phos) and ADPH-Oxidase reaction and through non-enzymatic process by forming Gluco oxidants and glycation (Evans, *et al*, 2002). Hyperglycemia is caused by abnormalities in insulin secretion or action of insulin disorders. The state of hyperglycemia in diabetes lead to increased formation of free radicals and anti-oxidants and a decrease in a number of events that eventually occurs is called oxidative stress. Hyperglycemia can induce an increase in free radicals through autooksidasi glucose, the formation of Advanced Glycation End products (AGEs), and increased polyol pathway activity (sorbitol) (Johansen *et al*, 2005)

The World Health Organization (WHO) estimates that 177 million people worldwide have diabetes and this number will increase to over 300 million by 2025. Indonesia in 2000 ranks fourth in the ranks of countries with the highest number of people with diabetes mellitus in the world after India (31.7 million), China (20.8 million) and the United States (17.7 million people). This figure is expected to rise to 21.3 million in 2030 (Wild, *et al*, 2004).

In patients with hyperglycemia were also found inflammatory reaction due to ease patients experienced an infection. Inflammation may increase the release of proinflammatory cytokines such as Tumor Necrosis Factor α (TNF-α). Besides an increase in TNF-α, a decline adinopektin that lead to insulin resistance. Prolonged insulin resistance pancreas cells no longer able to compensate insulin then there hyperglycemia. Hyperglycemia and release of excess free fatty acids would be material to the formation of triglycerides in the liver (Suastika, 2008).

The process of autooxidation on hyperglycemia and glycation reactions result in the release of electrons. The release of these electrons will trigger the formation of free radicals, particularly superoxide radicals ($^{\cdot}O_2$), and hydrogen peroxide ($^{\cdot}H_2O_2$) and via Haber-Weis and Fenton will form hydroxyl radicals ($^{\cdot}OH$). These materials are known as oxygen-free radicals (RBO), which can damage cell membranes, a lipid peroxides are known to malondialdehida (Tjokroprawiro, 1993).

Advanced Oxidation Protein Products (AOPP) are a group of compounds containing ditirosin, formed by cross-linking proteins (Alderman *et al*, 2002). Ditirosin molecule formation and cross linking the proteins associated with the formation of Advanced Glycation End Products (AGEs), the end product of non-enzymatic glycosylation reactions are complex and irreversible and can cause damage to proteins, lipids, carbohydrates, and ribose (Baynes, 1999). Based on research conducted by Suhartono (2005) has revealed that the administration of glucose 200 mg/dL in protein glycosylation cause reactions and compounds formed dikarbonil 7.5 times compared to the control. Similarly, incubation time in glycosylation reactions, can lead to increased formation dikarbonil compounds and advanced glycation end products (AGEs) were significantly, in addition to a decline in the levels of tyrosine.

Glycosylation reaction is the reaction between protein and glucose at high concentrations. This reaction is also called non-enzymatic glycation reactions or Maillard. This reaction is related to the mechanism of the occurrence of complications in diabetes mellitus which subsequently causes diabetic retinopathy, nephropathy, and atherosclerosis.

Is a non-enzymatic glycosylation reaction and is a complex reaction between reducing sugars and amine group on the protein. This non-enzymatic glycosylation occurs due to an increase in glucose levels continuously so hooking between glucose with primary amine group on the protein to be no longer reversible. These reactions may lead to further browning, fluorescence, and the formation of crosslinks (Soetmadji, 2001; Setiawan, 2005).

Pranajiwa (*Euchresta horsfieldii lesch benn*) seed extract has antioxidant effects that quite well with the percentage reduction above 50%, ie 82, 90%. These results prove that the seeds pranajiwa (*Euchresta horsfieldii lesch benn*) potentially increasing the capacity of antioxidants and reduce oxidative stress. After obtaining the active compounds, the continued testing using animal models such as hyperglycemia wistar rats in vivo induced by alloxan to determine the effectiveness of blood glucose levels drop and AGEs.

Generally, this study aims to determine the effectiveness of seed extract pranajiwa (Euchresta horsfieldii Lesch benn) in an effort to prevent damage to β-cells of the pancreas in alloxan induced hyperglycemic rat.

METHODS

Research Design

The study was a true experimental design with The Randomized Pre and Posttest Control Group Design (Pocock, 2008), is used to prove the effectiveness of seed extract pranajiwa (Euchresta horsfieldii Lesch benn) in β-cells to repair damaged pancreas by reducing blood glucose and AGEs in rats wistar hyperglycemia. The study used 40 Wistar rats were divided into four groups, one control group, and the three treatment groups (P1) dose of 0.5 mg /kg bw, the treatment group (P2) dose of 2 mg/kg bw and group (P3) dose of 5 mg/kg bw.

Pranajiwa (*Euchresta horsfieldii Lesch benn*) seed extraction

Pranajiwa its nut seeds cleaned and dried in the open air with circulating placed the open air and not exposed to direct sunlight. Furthermore, ground to a powder in a blender. Pranajiwa seed powder have weighed as much as 300 g dried and extracted by maceration using solvents methanol for 24 hours and then evaporated using a rotary vacuum evaporator. While the evaporated residue obtained re-extracted using methanol solvent. Methanol extract was evaporated to obtain thick seed extract and further pranajiwa diskrening phytochemicals to identify classes of compounds and determination of antioxidant capacity using DPPH (1,1-diphenyl-2-pikrilhidrazil). GC-MS analysis aimed to determine the active compounds contained in the seed extract pranajiwa potentially lowering blood glucose, and AGEs alloxan induced hyperglycemia. Extracts were investigated in this study is an extract that has a high potential based on preliminary test results. The research was conducted through maceration stage and GC-MS analysis was continued.

Treatment with Rats

A total of 40 wistar white rats aged 3 months were measured weight and fed a diet enriched standard formula for 1 month of vitamin B12. All rats adapted at home for 1 week. After all of the rats in the uniform condition then made hyperglycemic rats for 3 days by giving alloxan dose of 125 mg/kg bw, and then classified into 4 groups of experiments, the control group and the treatment group were each given seed extract at a dose pranajiwa 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day for seven weeks.

Laboratory Testing

Laboratory tests performed at the time of treatment until there is hyperglycemia, with testing procedures as follows:

a. Determination of blood glucose levels were measured in venous blood of rats with GLUKO test method.

b. Determination of AGEs levels were measured using the UV-Vis spectrophotometer analysis based on the formation of compounds dikarbonil via non-enzymatic glycosylation reactions.

e. Examination histopathological structure of pancreatic tissue wistar rats performed using binocular microscope with Gomori-Nuclear fast red staining, magnification 400x

Data Analysis

The data collected from this research was statistically analyzed by the following procedures. Statistical analysis was conducted using SPSS 13.0 application program for windows (Triton, 2006) for.

1. Normal distribution using Shapiro-Wilk within $\alpha = 0,05$;
2. Homogeneity of variance were analyzed using Levene's test to determine whether variations in respective homogeneous group.
3. Analysis of differentiation on mean levels increase of blood glucose, and decrease of AGEs from each group were analyzed using one-way ANOVA. Further analysis is one way anova Post Hoc Test; assuming homogeneous variance is then selected Post Hoc Test was LSD at significance level $\alpha = 0.05$.
4. Further t-paired test to see if each group before and after treatment gave significantly different results with a significance limit of $p<0.05$

RESULTS

Characteristics of Active Pranajiwa Seed Extract

Pranajiwa seed extract condensed methanol maceration results give as much as 32.55% yield blackish brown. While the results of antioxidant capacity against DPPH (1,1-diphenyl-2-pikrilhidrazin) showed that the seed extract has pranajiwa percentage reduction of 63.05% in 5 minutes and 82.90% within 60 minutes. Antioxidant capacity in the blood of Wistar rats was calculated based on the ability of catching free radical DPPH (1,1-diphenyl-2-pikrilhidrazil) with the capture reaction. The results of the separation of active compounds by GC-MS analysis showed that the seed extract compounds pranajiwa provides nine peaks with a retention time (tR), peak area (%), and has a different molecular weight. The compounds were detected in the methanol extract of pranajiwa seeds be presented in detail in Fig.1 are presented in Table 1

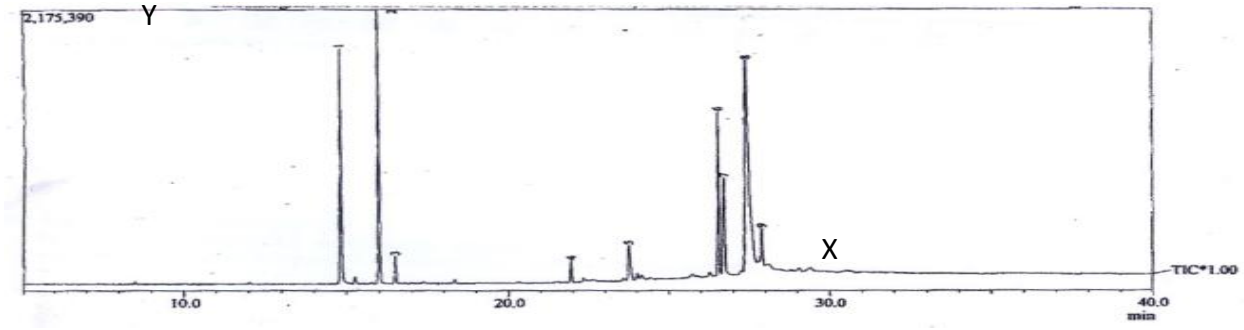

Figure 1. Seed Extract chromatograms Pranajiwa.

Table 1. The compounds were detected in the extract of seed pranajiwa.

Peak No	Retention time (Minutes)	Wide Area (%)	Molecular Formula	Compound Name
1	14.822	17.00	$C_{10}H_{12}O_2$	*Eugenol*
2	15.998	17.55	$C_{15}H_{24}$	*Trans-Caryophyllene*
3	16.495	1.67	$C_{15}H_{24}$	*α-humulene*
4	21.947	1.30	$C_{17}H_{34}O_2$	*Hexadecanoic acid*
5	23.747	3.66	$C_{19}H_{34}O_2$	*9,12-octadecadienoic acid*
6	26.539	10.06	$C_{22}H_{42}O_4$	
7	26.716	8.45	$C_{15}H_{24}$	*Hexanedioic acid*
8	27.408	38.02	N_2O	*Matrine*
9	27.891	2.30	**BM = 302**	**New Compound**
			$C_{24}H_{38}O_4$	*1,2-benzene dicarboxylic acid*

Description: The results of spectroscopic analysis QP2010S Shimadzu GC-MS

Blood Glucose Levels Decrease Hyperglycemia Wistar Rats

Data mean blood glucose levels of hyperglycemia Wistar rats both pre and posttest is presented in Table 2. While the profile of blood glucose levels before and after treatment of various doses pranajiwa seed extract is presented in Figure 2.

Table 2. Blood Glucose Levels Before and After Treatment.

Treatment	Observations blood glucose levels (mg / dL)		
	Pretest The mean ± SD	Posttest The mean ± SD	Difference of Blood Glukose Levels (mg/dL)
Control (K-) and	219,35 ± 3,06	110,76 ± 2,28	108,59 [b]
(K+) Extract dose	218,56 ± 3,19	138,59 ± 3,54	79,97 [d]
0,5 mg	217,43 ± 2,07	128,39 ± 1,71	89,04 [c]
Extract dose 2 mg	217,61 ± 2,77	103,69 ± 1,88	113,03 [a]
Extract dose 5 mg			

Note: Difference in average values followed by different letters in the same column, shows the test results were significantly different (p<0.05) LSD test for posstest

K-(Negatitve Control)

K + (Positive Control/Glibenclamide)

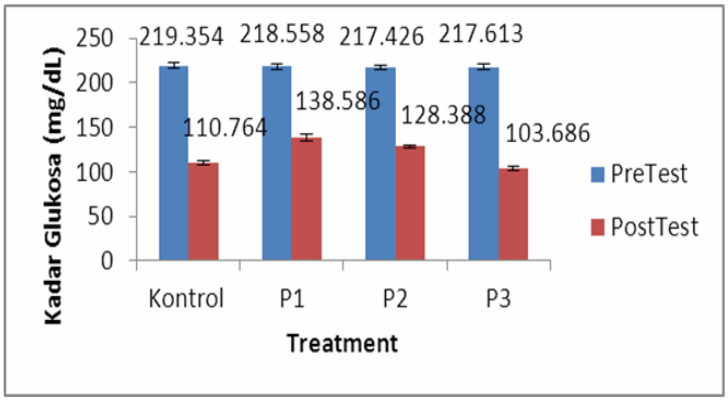

Figure 2. Profile of Blood Glucose Levels before and After Treatment.

Decrease of AGEs Levels Wistar rats Hyperglycemia

The AGEs levels hyperglycemia pre and posttest are presented in Table 3. Profile AGEs levels before and after treatment with various doses pranajiwa of seed extract is presented in Figure 3.

Table 3. Levels of AGEs Before and After Treatment.

Treatment	Observations AGEs levels (mol/L)		
	Pretest The mean ± SD	Posttest The mean ± SD	Difference of AGEs Levels (mol/L)
Contol K- and K+	0,223 ± 0,01	0,049 ± 0,01	0,174 [b]
Extract dose 0,5 mg	0,221 ± 0,01	0,089 ± 0,01	0,132 [d]
Extract dose 2 mg	0,205 ± 0,01	0,067 ± 0,01	0,138 [c]
Extract dose 5 mg	0,207 ± 0,01	0,038 ± 0,01	0,169 [a]

Note: Difference in average values followed by different letters in the same column, shows the test results weresignificantly different (p<0.05) LSD test for posstest.

K-(Negative Control)

K+ (Positif Control/Glibenclamide)

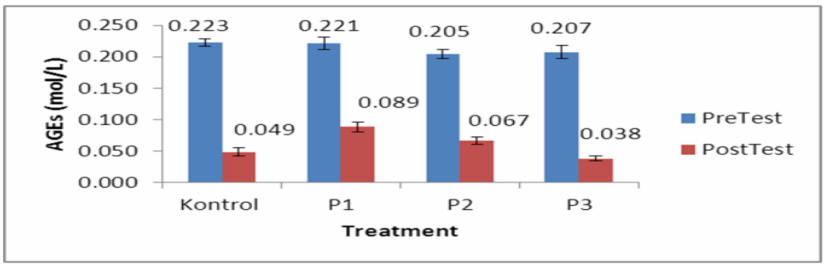

Figure 3. Profile of AGEs Levels before and After Treatment.

Test results with the Shapiro-Wilks normality and homogeneity test with Levene's test shows that the data mean blood glucose levels and of AGEs levels Wistar rats before and after administration of various pranajiwa seed extract showed dose throughout the data were normally distributed and homogeneous variants (p>0,05).

Results of analysis and one way ANOVA followed by LSD test showed that there were significant differences between the levels of AGEs Wistar rat control group (K+) treatment group after the administration of seed extract pranajiwa 0.5 mg/kg bw, 2 mg/kg bw and 5 mg/kg bw with a value of p<0.05. The results also showed that the control group (K+) gives the equivalent effect of the treatment group a dose of 5 mg/kg bw.

Furthermore, the limit of significance with paired t-test showed a significant difference in the mean decrease in AGEs levels between the control group (K-) with control group (K+) with p<0.05. In contrast, the treatment group a dose of 0.5 mg/kg bw dose group treated with 2 mg/kg bw bothwere significantly different (p<0.05), while the treatment group a dose of 5 mg/kg bw occur both significant differences with p<0.05.

Structure of Histopathology Pancreas Network Wistar rats

Gomori-Nuclear fast red staining done to see qualitative changes in the structure of rat pancreatic tissue treatment.

Staining is composed of two color components, and the Gomori-Nuclear fast red. Gomori an alkaline dye in order to color the cell nucleus that are acidic while Nuclear fast red is an acidic dye that can stain the cytoplasm is alkaline.Histopathological changes in pancreatic tissue morphology Wistar rats with 400 times magnification and staining Gomori-Nuclear fast red from the normal state to occur alloxan-induced hyperglycemia caused a dose 125 mg/kg bw can be seen in Figure 4 and 5.

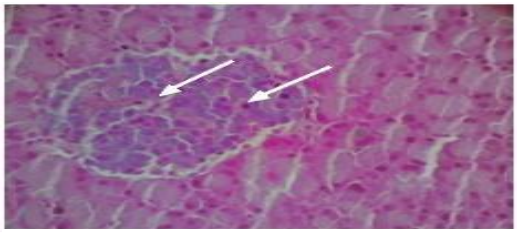

Figure 4. Histopat Normal Histopat Pancreas Wistar Rats (Magnification 400x)

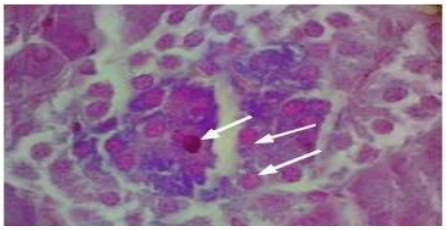

Figure 5. Histopat Normal Histopat Pancreas Wistar Rats K- (alloxan)(Magnification 400x)

Figure 4 shows that the number of cytoplasmic granules Wistar rats normally looks still intact, no visible presence of clinical symptoms, and found no β cell nuclei and other cell degeneration to necrosis around the islets of Langerhans in mikroskpis examination, both qualitatively and quantitatively compared control group (K-and K+) and the treatment group. In contrast to Figure 5 shows that the pancreatic β-cells were detected by staining with Gomori-Nuclear fast red shown in the figure are colored purple cytoplasmic granules. The loss of a number of cytoplasmic granules around the islets of Langerhans. Rupture of a number of β-cell nuclei (karyoreksis), shrinking the cell nucleus and no visible piknosis clear cell boundaries between β-cells and α-cells around the islands of Langerhans. Wistar rat pancreatic β-cell degeneration to necrosis caused by alloxan induced a dose 125 mg/kg bw more than the Wistar rat pancreatic β-cells in the treatment group. This is because alloxan is selectively destroy pancreatic β-cells through the formation of reactive oxygen species that begins by alloxan reduction and characterized by elevated blood glucose levels (hyperglycemia). Nowhere β-cells around the islets of Langerhans beta cells than in normal Wistar rats. Stroma reduced density of Langerhans on the island, there is edema, congestion, to undergo necrosis (cell death).

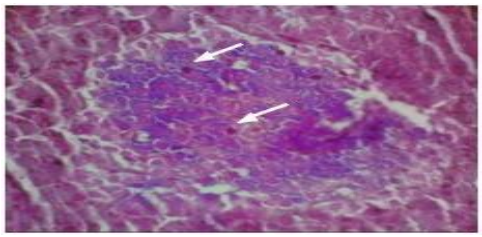

Figure 6 Histopat Pancreas Wistar Rats dose of 0.5 mg/kg bw (Magnification 400x)

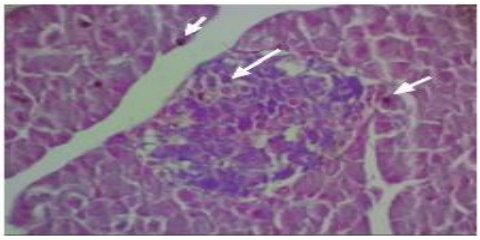

Figure 7. Histopat Pancreas Wistar rats Dose 2 mg/kg bw (Magnification 400x)

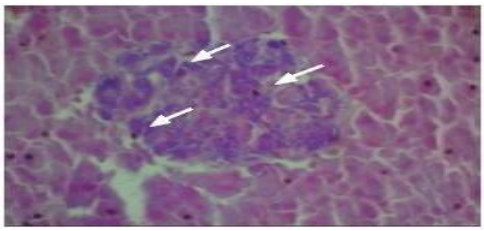

Figure 8. Histopat Pancreas Wistar Rats dose of 5 mg/kg bw

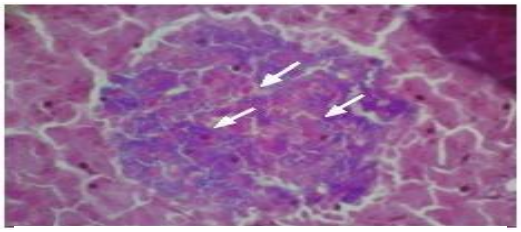

Figure 9 Histopat Pancreas Wistar Rats K+ (Glibenclamide)

In Figure 6 above have been changes in pancreatic tissue morphology histopathology in Wistar rat islets of Langerhans due pranajiwa seed extract dose of 0.5 mg/kg bw compared with the negative control group (K-), although the amount of pancreatic β-cell degenerating to necrosis rather reduced. This means pranajiwa seed extract dose of 0.5 mg/kg bw cannot help the process of pancreatic tissue repair damage caused by alloxan induced. In contrast to Figure 7 shows that employment pranajiwa seed extract dose of 2 mg/kg bw to changes in pancreatic tissue morphology structure is to stimulate cell division. There is an increasing amount of pancreatic β-cells means in accordance with the theory that when the cells are injured due to something so potentially stimuli undergo reversible changes that can be back to normal. Mechanisms of pancreas due to improved seed extract pranajiwa dose of 2 mg/kg bw is likely pranajiwa seed extract dose of 2 mg/kg bw contains large insulincompare treatment groups in a dose of 0.5 mg/kg bw resulting in destruction of pancreatic β-cells quickly and normal.Gambar nearing still visible above the cells undergoing necrosis.

Figure 8 does not appear on any cell degeneration to necrosis of the Wistar rat pancreatic tissue around the islands of Langerhans thus showing clear boundaries between β-cells by alpha cells. Similarly, the amount of cytoplasmic granules in the beta cell nucleus has increased to near-normal conditions so that the pancreatic tissue repair process can take place quickly. In contrast to Figure 9 morphological changes in the structure of the network in the Wistar rat pancreatic islands of Langerhans due antidiabetic drug administration (Glibenclamide) as a posttest control. No visible cytoplasmic granules and clear boundaries between beta cells by α-cells, still seems the cells undergoing necrosis and pancreatic β-cell repair process is not perfect.

Carried observation of β-cells quantitatively by calculating the number of β-cells in the Wistar rat pancreatic tissue of each group and the control group both treatment groups. β-cells were detected by staining with Gomori-Nuclear fast red and 100 times magnification images of cells are shown in blue on the islets of Langerhans cell while the other is red. Results of calculating the number of β-cells in pancreatic islets of Langerhans Wistar rats in the control group and the treatment group fifths of the field of view is presented in Table 4

Table 4. The average number of β-cells in the islets of Langerhans Wistar rat pancreatic tissue.

Treatment	**The number of β-cells in the of Langerhans (Fruit)**
	The mean ± SD
Normal control	60,12± 4,98 [f]
Negative control (K-)	5,44 ± 1,83 [a]
Positivecontrol (K+)	32, 84± 5,30 [d]
Pranajiwa seed extract dose 0,5 mg/kg ww	9,04 ± 2,13 [b]
Pranajiwa seed extract dose 2 mg/kg ww	13,48 ± 4,04 [c]
Pranajiwa seed extract dose 5 mg/kg ww	41,52 ± 6,48 [e]

Catatan: Difference in average number of β-cells by different letters in the same column, shows the test results were significantly different (p <0.05) LSD test.

K-(Negative Control)

K+ (Positive Control / Glibenclamide)

DISCUSSION

Characteristics pranajiwa (*Euchresta horsfieldii lesch benn*) seed extract

The process of solvent extraction with methanol is intended to get all the components of polar or easily soluble in water from the sample because it has OH bond. Methanol extracts pranajiwa seed that has a very high antioxidant capacity and have the ability to destroy cells, dissolving the bioactive compounds as well as to maintain the reactivity properties of a compound. According to Voigt (1995) the ability of a solvent to extract the contents of the cell is affected by its ability to loosen the cell walls of cellulose framework and dissolve the active components of the cell contents. This is done in line with the objectives of the study, namely the effectiveness pranajiwa seed extract (*Euchresta horsfieldii lesch benn*) to prevent damage to pancreatic β-cells in mice hyperglycemia. In essence, to look for a compound that can later be applied as a remedy, at least these compounds should be easily soluble in water (body fluid) that can provide direct therapeutic effect (Rang *et al*, 1987).

The compound α-humulene is one class of terpenoid derivatives of compounds derived from seed extract as a natural antioxidant potential pranajiwa will release the H atom (hydrogen) atoms bound to O (oxygen) to the-OH (hydroxyl) radicals forming. Furthermore, H radicals would react with 1,1-diphenyl-2-pikrilhidrazil form a 1.1-diphenyl-2-pikrilhidrazin. Reaction formation of 1,1-diphenyl-2-pikrilhidrazin will cause a reduction DPPH absorbance (Djatmiko, 1998; Sofia, 2009).

Skrening test is used to identify the class of phytochemical compounds present in seed extracts pranajiwa use various detection reagents. Phytochemical test results with reagent-Buchard Lieberman indicated that the methanol extract of condensed positive pranajiwa seeds contain compounds suspected group terpenoid compound α-humulene and trans-caryophyllene. Alkaloid of the reagent test showed a white precipitate Meyer and Wagner reagent precipitates a brown with a distinctive color intensity suspected compound matrine. Pranajiwa seed extract also contains phenolic compounds against reagent FeCl3 group that gives color change from orange-red to purple-black the alleged compound eugenol, and identified flavonoid compounds with wilstater reagent that gives the red color to yellow orange brick (Harborne, 1987; Tiwari et al , 2011). This can be proved by the results of GC-MS spectroscopic analysis.

The results of GC-MS spectroscopic analysis of the seed extract has been detected pranajiwa 9 components implies the active compounds in the top 1% of the area namely, eugenol (17%) are the class of phenolic compounds, trans-caryophyllene (17.55%), and α-humulene (1.67%) belongs to the class of terpenoid compounds, while Hexadecanoic acid (1.30%), 9.12-hexadecadienoic acid (3.66%), hexanedioic acid (10.06%) and 1,2-benzenedicarboxylic acid (2.30%) is a carboxylic acid group (phenolic). Other active compounds detected is compound matrine (8.45%) is a class of alkaloid compounds and detected one new compound (38.02%) with a molecular weight of 302.

α-humulene compounds derived from seed extract pranajiwa included into the class of sesquiterpene compounds which act as natural antioxidants and antihiperglikemia (Moss, 2011). According to Corey (2002), α-humulene compound has the ability to reduce the hydroxyl radical (OH) and is a form of the hormone insulin produced in the body that works by lowering levels of glucose in the blood (Athanasios *et al*, 2009).

Similarly, 1,2-benzenedicarboxylic acid compounds have the ability to reduce levels of AGEs. The compound is a phenolic derivative that can act as a proton donor, which releases hydrogen atoms and are able to scavenge free radicals formed from the reaction of non-enzymatic glycosylation end products through the reaction between the aldehyde group of carbohydrates and amino groups of proteins (Soetmadji, 2001; Firdus *et al*, 2004).

Analysis of Blood Glucose Levels in Wistar rats Hyperglycemia

In this study it was found that the extract seeds pranajiwa with various doses can lower blood glucose levels in hyperglycemic rats wistar. Data rates of hyperglycemia mice blood glucose levels are presented in Table 2. The results of statistical analysis showed that the administration of alloxan could cause an increase in blood glucose levels. This is in accordance with the opinion of Williams (1991) that the administration of alloxan can cause damage to β-cells of Langerhans islands which can cause hyperglycemia. Alloxan will reduce insulin production that can cause an increase in blood glucose levels or hyperglycemia. Increased blood glucose levels also caused because of the degeneration of the pancreatic β-cells causes impaired insulin production resulting in insulin deficiency. The decrease of insulin causes glucose consumed whole body cannot be perfectly processed, resulting in the body's blood glucose levels rise (Greenspan, 1998).

From the same table can also be seen the average blood glucose levels wistar rats hyperglycemic positive control group posttest between the treatment groups at pranajiwa seed extract with various doses of 0 mg/kg bw/day, 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day. The difference in the mean reduction in blood glucose levels due pranajiwa seed extract with various doses of 0 mg/kg bw/day, 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day can be seen in Table 3. From the table it obtained the highest blood glucose levels decrease occurred in pranajiwa seed extract dose of 5 mg / kg bw/day in the amount of 34.90 mg/dL

Mechanism of action as antihiperglikemia pranajiwa seed extract in lowering glucose levels mainly played by the compound α-humulene through expenditure-insulin by pancreatic β-cells or alter the metabolism of glucose. α-humulene role in increasing insulin secretion by β-cells through a mechanism of pancreatic beta cells to maintain a functioning and repair work so that insulin secretion by pancreatic β cells of Langerhans islands may increase (Moss, 2011). Subroto (2005) revealed that red fruits can control blood glucose levels. There are two mechanisms performed in the treatment of diabetes that leads to the production of insulin and inhibit the action of the enzyme alpha-glycosidase where these enzymes play a role in degrading carbohydrate taken into the body and converted into glucose. When the work of the alpha-glycosidase enzyme can be inhibited, the conversion of carbohydrates into glucose can be reduced, so that the blood glucose lowering effect.

Analysis of AGEs levels in Wistar Rats

Pranajiwa seed extract with various doses can lower blood levels of AGEs wistar rats hyperglycemia. Data average levels of AGEs can be seen in Tabel.3. Mean blood levels of AGEs hyperglycemic rats in the negative control group (K-) and positive control group (K+)was 0.223 ± 0.01 mol /L and 0.049 ± 0.01. Mol/L. From the same table can also be seen average levels of AGEs in wistar rats in the extract at a dose of 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day in a row for the pre and posttest was (0.221 ± 0.01), (0.205 ± 0.01), (0.207 ± 0.01) mol/L and (0.089 ± 0.01), (0.067 ± 0.01) and (0.038 ± 0, 00) mol/L.

From the data obtained mean difference decreased levels of AGEs in the highest dose of 5 mg/kg bw/day in the amount of 0.05 mol/L which showed a statistically significant with $p<0.05$ compared to the control group (glibenclamide) and treatment group on dose of 0.5 mg/kg bw/day and the dose of 2 mg/kg bw/day. Ability pranajiwa seed extract lowers levels of AGEs played by the content of active compounds, namely 1,2-benzenedicarboxylic acid. The compound is a phenolic derivative that can act as a proton donor, who releases hydrogen atoms and are able to scavenge free radicals formed from the reaction of non-enzymatic glycosylation end products through the reaction between the aldehyde group of carbohydrates and amino groups of proteins (Soetmadji, 2001; Firdus *et al*, 2004).

In the above results can be explained that patients with prolonged hyperglycemia glycosylation process occurs in a long time led to high levels of AGEs. Glycosylation process causes the formation of products amadori. Amadori products will form advanced glycosylation end-products (AGEs) and protein kinase C pathway in patients with obesity and an increase in free fatty acid (FFA). Cumulatively over the state will increase the formation of free radicals through the mitochondrial electron transport system, polyol pathway and FFA (Srivastava, 2005).

A high level of AGEs in the blood is one of the important factors complications of diabetes mellitus. Root cause of AGEs in patients with hyperglycemia is high glucose levels or the fluctuation of blood glucose levels continuously. In addition due to the levels of blood glucose, AGEs also increase oxidative stress in the event. Oxidative stress is a condition in which the levels of endogenous antioxidants are not able to cope with the free radicals that are formed in the body. Free radicals will accelerate the formation of AGEs, and AGEs otherwise would increase the formation of free radicals (Bucala *et al*, 1995; Turk, 2001).

Network Structure Changes in Histopathology Pancreas

Pancreatic tissue histopathology test results on wistar rats under normal circumstances indicate that the number of cytoplasmic granules seen many and not show clinical symptoms, β cell nucleus is still intact, and not degenerate until necrosis around the islets of Langerhans both qualitatively and quantitatively compared to the control group as well as the treatment group.

After alloxan induced a dose 125 mg/kg bw showed that pancreatic tissue morpologis changes, the cells did not show a clear boundary between beta cells and alpha cells to a reduced number of cytoplasmic granules around the islands of Langerhans. Wistar rat pancreatic beta cell degeneration to necrosis (cell death). This is caused by alloxan can form damaging free radicals and cell membrane permeability resulting in damage to β-cells of the pancreas that produce insulin function. According to Aronson (2008) hyperglycemia can worsen the destruction of beta cells. The reason, chronic hyperglycemia conditions tend to increase the formation of free radicals (ROS) such as glucose metabolism through autooksidasi glucose metabolism metilglioksal formation, and oxidative phosphorylation. Toxic action of alloxan on pancreatic cells-β is initiated by free radicals formed by redox reactions. Action of free radicals with high stimulation increases cytosolic calcium concentration that causes destruction of pancreatic beta cells Accept. Increased cytosolic calcium concentration also caused by alloxan induces mitochondrial calcium spending which led to disruption of the oxidation of pancreatic cells-β (Borg *et al*, 1979; Watkins *et al*, 2008). In pranajiwa seed extract dose of 5 mg/kg bw/day did not appear any cells undergoing necrosis in wistar rat pancreatic tissue around the islands of Langerhans.

Similarly, the number of beta cells have increased to near-normal conditions that pancreatic tissue repair process can take place quickly. It is played by α-humulene compounds through expenditure-insulin by pancreatic β- cells or alter the metabolism of glucose in the cells increases insulin secretion by pancreatic β-through mechanism in maintaining a functioning beta cells and α-humulene compounds having reactive groups on the molecular structure , resulting in the ability to capture free radicals are also getting stronger, so the pancreatic cell-β damage and changes in the structure of β-pancreatic tissue histopathology in rats wistar patients with hyperglycemia can be corrected.

CONCLUSION AND RECOMMENDATIONS

1. Pranajiwa seed extract (Euchresta horsfieldii Lesch benn) at a dose of 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day can lower blood glucose levels, and of Advanced Glycation End-Products, in rats wistar alloxan induced hyperglycemia
2. Pranajiwa seed extract (*Euchresta horsfieldii lesch benn*) dose of 5 mg/kg bw/day can give best effect to the reduction of blood glucose levels and AGEs in wistar rats alloxan-induced hyperglycemia. Decrease in blood glucose levels by 34.90 mg/dL, decreased levels of AGEs by 0,05 mol/L mol/L
3. Pranajiwa seed extract (*Euchresta horsfieldii lesch benn*) improve the structure of histopathological tissue of wistar rat pancreatic β-cell damage caused by alloxan induced dose of 125 mg/kg bw.

SUGGESTION

1. Need further research on the role of seed extract pranajiwa (Euchresta horsfieldii Lesch benn) against biomarkers include TNF-α, IL-6, and other cytokines.
2. Finding the relationship between decreased levels of blood glucose and AGEs with reduced due pranajiwa seed extract.
3. Further research needs to be done in the form of clinical trial stage for human subjects hyperglycemia, whether pranajiwa seed extract have the same effect.

ACKNOWLEDGMENTS

On this occasion, the researcher would like to thank the University of Udayana for financial support through grants BOPTN Featured Colleges with Contract Number: 175A.19/UN14.2/PNL.01.03.00/2013.

REFERENCES

Aronson, D. 2008. Hyperglycemia and the pathobiology of diabetic complications. *Adv. Cardiol.* 45: 1-16.

Astuti, S.N. 2003, Uji Pendahuluan Efek Aphrodisiak Ekstrak Biji Pranajiwa Manis (Euchresta javanica R. Br) pada Tikus Putih jantan Dewasa. (Skripsi), Surabaya; Universitas Widya Mandala.

Alderman, C.J.J., Shah S., Foreman, J.C., Chain, B.M. and Katz, D.R. 2002. The role of advanced oxidation protein products in regulation of dendritic cell function, *Free Rad. Biol. Med*, 32: 377-85

Budianto, R., Setiawan, B. and Suhartono, E. 2003., Potensi Antiradikal in vitro infus tanaman pasak bumi (Eurycoma longifolia Jack) dengan menggunakan model reaksi Fenton, Disampaikan pada seminar nasional Teknik Kimia Unika Parahyangan, Bandung.

Corey, E.J. 2002, "Short Syntheses of (±)-δ-Araneosene and Humulene Utilizing a Combination of Four-Component Assembly and Palladium-Mediated Cyclization". Organic Letters 4 (14): 2441–2443. doi:10.1021/ol026205p. PMID 12098267.

Evans, J.L., Goldfine, I.D., Maddux, B.A., and Grodsky, G.M. 2002., Oxidative Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes, Endocrine Review 23: 599-622

Heyne, K. 1987. Tumbuhan Berguna Indonesia III, terjemahan : Badan Penelitian dan Pengembangan Kehutanan, Yayasan Sarana Wana jaya, Jakarta

Ihnat, M.A., Kaltreider, R.C., Thorpe, J.E., Green, D.E., Kamat, C.D., Leeper, M., Shanner, A.C., Warnke, L.A., Piconi, L., and Ceriello, A. 2007., Attenuated Superoxide Dismutase Inductionin Retinal Cells in Response to Intermittent High Glucoseversus High Glucose, *Am J. Soc. of Biochem and Biotech*, 3(1):16-23

Johansen JS, Harris AK, Rychly DJ, Ergul A. 2005., Oxidative stress and the use of antioxidants in diabetes: lingking basic science to clinical practice. Cardiovasc Diabetol. April 29: 4(5). Available from URL: http://www.pubmedcentral.nih.gov/tocrender.fcgi?iid=18041

Murthy, U.M., and Sun, W.Q. 2000., Protein modification by Amadori and Maillard reaction during seed storage: roles of sugar hydrolysis and lipid peroxidation. *J Exp Botany*. 51 (348): 1221-8.

PERKENI. 2012., Konsensus Pengelolaan Diabetes pada Diabetes Melitus tipe 2. PB Perkeni. Jakarta.

Pocock, S.J. 2008, Clinical Trial a Practical Approach, Chichester-New York, Singapore: Jon Wiley & Son Ltd

Setiawan B. 2005, Model pembentukkan advanced glycation end products dan modifikasi protein akibat reaksi glikosilasi, *Maj. Kedokt. Indon*, 55(11) : 681-85

Soetmadji, D.J. 2001. The role of free radical in management of type 2 diabetic patiens. Disampaikan pada symposium Free Radicals in Diabetes and their interaction with Sulphonylurea, Jakarta.

Suastika. 2008, Obesitas Sindrom Metabolik Diabetes Dislipidemia Penyakit Tiroid, Kumpulan Naskah Ilmiah, Udayana University Press, ISBN : 978-79-8286-56-8

Triton, P.B. 2006. SPSS 13.0 Terapan: Riset Statistik Parametrik, Yogyakarta: Andi, hal 61-108

Turk, Z. 2001. Glycation and AGEs in diabetes and complications. EJIFCC. 13:5.

Murthy, U.M., and Sun, W.Q. 2000. Protein modification by Amadori and Maillard reaction during seed storage: roles of sugar hydrolysis and lipid peroxidation. *J Exp Botany*. 51 (348): 1221-8.

PERKENI. 2012. Konsensus Pengelolaan Diabetes pada Diabetes Melitus tipe 2. PB Perkeni. Jakarta.

Pocock, S.J. 2008. Clinical Trial a Practical Approach, Chichester-New York, Singapore: Jon Wiley & Son Ltd.

Setiawan B. 2005. Model pembentukkan advanced glycation end products dan modifikasi protein akibat reaksi glikosilasi, *Maj. Kedokt. Indon*, 55(11) : 681-85

Suastika. 2008. Obesitas Sindrom Metabolik Diabetes Dislipidemia Penyakit Tiroid, Kumpulan Naskah Ilmiah, Udayana University Press, ISBN : 978-79-8286-56-8

Suhartono E., Setiawan B., Edyson. 2005. Uji Aktivitas antioksidan jus buah mengkudu (Morinda citrifolia) dan perannya sebagai inhibitor Advanced Glycation End Products (AGEs), 37(1) : 1-6

Turk, Z. 2001. Glycation and AGEs in diabetes and complications. EJIFCC. 13:5

Ulrich, P., Cerami, A. 2001. Protein Glycation, diabetes, and aging, *J Clin Invest*. 1-4

Wild, S., Roglic, G., Green, A., Sicree, R., King, H. 2004., "Global Prevalence of Diabetes estimates for the year 2000 and projections for 2030", Diabetes Care, Vol 27, No. 5 May 2004, pp. 1047-53.

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