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POTENTIAL OF EUCHRESTA HORSFIELDII LESCH BENN LEAF EXTRACT PREVENT OXIDATIVE STRESS THROUGH DECREASE OF MALONDIALDEHYDE LEVELS AND PROFILE HISTOPATHOLOGY PANCREATIC B-CELLS IN DIABETIC RATS

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ABSTRACT
Oxidative stress is damage to cells caused by chemical reactions between free radicals and molecules in the body. Cell damage caused by oxidative stress is believed to be the cause of various diseases such as diabetic. As a result, the intensity of the oxidation process of normal body cells becomes higher and cause more damage. The results showed that the Euchresta horsfieldii lesch ben leaf extract can prevent oxidative stress due to the production of compounds reactive oxygen species (ROS), thereby protecting cells from damage functions. Oxidative stress can be formed through decreased malondialdehyde levels in the body, then carried out research with the aim to prove that the n-hexane extract of leaves Euchresta horsfieldii lesch benn has the ability as through decreased malondialdehyde levels of antioxidative and histopathological profile improvement of pancreatic β-cells in diabetic rats. This study is a laboratory experimental study design was randomized pre and post-test control group design. A total of 36 rats were divided into four groups: one control group and three groups treated with different theEuchresta horsfieldii lesch benn leaf extract that each dose of 0.5 mg/kg bw, 2 mg/kg bw and 5 mg/kg bw. All groups induced by alloxan dose of 140 mg/kg bw in order to get a diabetic condition. After treatment for 8 weeks, blood samples were taken for examination malondialdehyde levels. The results showed that the Euchresta horsfieldii lesch benn leaf extract can prevent oxidative stress by decreasing the average levels of MDA in the positive control group (Glibenclamide) of (0.61 ± 0.07) µmol/L, the treatment groups respectively P1 = (0,96 ± 0.08) µmol/L; P2 = (0.72 ± 0.08) µmol/L; and P3 = (0.46 ± 0.06) µmol/L of different statistic significant with $p < 0.05$.

KEYWORDS: Euchresta horsfieldi lesch benn, Oxidative Stress, malondialdehyde, Diabetic.

INTRODUCTION
Cardiovascular complications in diabetes mellitus is one of the factors causing oxidative stress at the cellular level. Although the administration of insulin or oral antidiabetic drugs, complications of diabetes is still difficult to overcome almost all over the world, including Indonesia. Deaths due to these complications is estimated at 40% of patients with diabetes mellitus. This situation triggered by failure of the pancreatic β-cells in the production of insulin and glucagon, followed by high plasma (Srinivasan, 2007; Suastika, 2008).

Various complications can be caused by poor control of diabetes. The complications which include systemic vascular disease (accelerated atherosclerosis), heart disease, microvascular disease of the eye as a cause of blindness and retinal degeneration (diabetic retinopathy), cataracts, kidney damage as a cause of kidney failure and peripheral nerve damage (diabetic neuropathy). The extent of complications in diabetes seems to be correlated with blood glucose concentration so that excess glucose is thought to be a major cause of tissue damage (Rahbani *et al*, 1999; Halliwell *et al*, 1999). This phenomenon can be caused by the ability of hyperglycemia in vivo in the oxidative modification of various substrates. In addition, hyperglycemia is also involved in the formation of free radicals (Droge, 2002). Hyperglycemia causes glucose autooksidasi, glycation of proteins, and activation of metabolic pathways polyols which further accelerates the formation of reactive oxygen species (Ueno, 2002). The formation of reactive oxygen compounds that can improve lipid modification, DNA, and proteins in different tissues. Molecular modifications in various tissues has resulted in an imbalance between antioxidant protective (antioxidant defense) and increased production of free radicals. It was the beginning of oxidative damage known as oxidative stress (Nuttal *et al*, 1999). Oxidative stress is a

pathological condition caused by damage to tissues in the body because of the increased number of free radicals that are not normal. The existence of these free radicals can be known through MDA as lipid peroxidation products.

Diabetes mellitus is a multifactorial disorder associated with proinflammatory cytokines that are characterized by the formation of compounds of reactive oxygen species (ROS) excess (Sahebari *et al*, 2011). ROS is part of the free radicals which are the product of normal cell metabolism. Free radicals are one of the products of chemical reactions in the body in the form of atoms or groups that have unpaired electrons on the outer orbital, so that these compounds are highly reactive (unstable). In the body, free radicals can cause lipid peroxidation process (Favier., 1995). Lipid peroxidation is the oxidative destruction of the unsaturated fatty acid chain length (Polyunsaturated Fatty Acids) that produce compounds of malondialdehyde (MDA). Thus, MDA can be used as an index measuring the activity of free radicals in the body. High levels of MDA in the body can be caused by increased activity of free radicals (Haliwell., 1999).

Malondialdehyde an end product of lipid peroxidation, which is usually used as a biological biomarkers of lipid peroxidation and describes the degree of oxidative stress (Hendromartono, 2000). According Suryohudoyo (2000), MDA is dialdehydes compounds or three reactive carbon is the final product of lipid peroxidation in cell membranes. MDA in biological material present in free form or form a complex bond with other elements in the network.

MDA measurements carried out by the researchers as an indirect index of oxidative damage caused by lipid peroxidation. According to a statement Tokur *et al*. (2006), MDA measurement principle is the reaction of one molecule of MDA with two molecules tiobarbiturat acid (TBA) form a complex compound of the MDA-TBA pink and quantity can be read with a spectrophotometer. These conditions lead to oxidative stress, ie an imbalance between free radicals with antioxidants in the body causes changes in pancreatic tissue histology and increased levels of malondialdehyde. However, in anticipation of the buildup of excess free radicals, it is necessary given the exogenous antioxidants from natural sources, one of which is the n-hexane Euchresta horsfieldii lesch benn leaf extract.

Kloppenburgh, (2006), reported that one of the traditional medicinal plants that have the potential to be developed as an antidiabetic drug leaves Euchresta horsfieldii lesch benn, because traditionally leaves Euchresta horsfieldii lesch benn has been used to treat various diseases, such as: diabetic, asthma, coughing up blood, and a dry cough. Other uses are as an antidote to snake venom, alfrodisiakum, and induce vomiting due to food poisoning. The results showed that the Euchresta horsfieldii lesch benn leaf extract contains several chemical compounds, including; α-humulene, trans-caryophyllene, eugenol, 1,2-benzene dicarboxylic acids, phenolic acids and a new compound with a molecular weight (m/z) 302. The compound α-humulene sesquiterpenoids included in the class, a compound known to have activity as an antioxidant and repair function of pancreatic β-cell destruction through decreased levels of blood glucose and AGEs in Wistar rats hyperglycemia (Gunawan, 2013). According Astuti (2003) Euchresta javanica R Br seed extract in male rats can increase superoxide dismutase and lower levels of malondialdehyde, so it can protect cells against oxidative stress.

RESEARCH METHODS

Research design

This study was a laboratory experimental design with The Randomized Pre and posttest control group design (Pocock, 2008). The sample in this study are male rats, aged 3 months were obtained center study of animal diseases Faculty of Veterinary Medicine Udayana University. The sample size in this study was 36 Wistar rats were classified into four groups: one control group and three treatment groups.

The initial step is to create uniform conditions of rats then 36th rats fed a diet try ITB rich in vitamin B12 for 4 weeks. Furthermore, to get the mice with diabetes are given alloxan dose of 140 mg/kgbw for 2-3 days. Each group was given the Euchresta horsfieldii lesch benn leaf extract known antioxidant capacity with various doses of 0.5 mg/kg bw, 2 m/kg bw, and 5 mg/kgbw for 8 weeks. Later examination malondialdehyde levels and see the profiles of pancreatic β-cells histopathology rats.

Euchresta horsfieldii lesch benn Leaf Extraction

The Euchresta horsfieldii lesch benn leaf which has been cleaned and then dried in the open air with open air circulation and is not exposed to direct sunlight. Subsequently milled to a powder blender. Euchresta horsfieldii lesch benn leaf powder that was dried weighed 1 kg and was extracted by maceration using n-hexane for 24 hours and then evaporated using a rotary vacuum evaporator. While the residue obtained re-extracted using n-hexane. n-hexane extract is evaporated to obtain a thick extract n-hexane and subsequently under taken phytochemical test and analysis Gas Chromatography Mass Spectroscopy (GC-MS) to determine the class of compounds in pranajiwa seed extract.

Treatment with Rats

A total of 36 male rats aged 3 months were measured weight and given a standard diet enriched formula of vitamin B12 for 1 month. All mice in cages adapted for 1 week. After all the mice in uniform conditions, the rats were made diabetic for 3 days by means of induced alloxan dose of 140 mg / kg, then made four experimental groups: one control group and three

treatment groups were each given Euchresta horsfieldii lesch benn leaf extract dose of 0,5 mg/kg, 2 mg/kg and 5 mg/kg for 8 weeks. Euchresta horsfieldii lesch benn extract is done by dissolving the extract into 5 ml of distilled water, then homogenized and exposed to mice orally in accordance treatments by disonde.

After the 8-week treatment period was terminated rats (euthanasi) with CO_2 done quickly and sterile. Pancreatic tissue is taken and washed with Phosphate Buffer Saline (PBS), drained and weighed, then packed with alufo and stored in freeser (-20°C) is then performed histopathological examination of the rat pancreatic tissue.

Blood Examination

Rats will have blood drawn fasted for 12 hours but drinking still be given as usual, then the mice weighed. After anesthesia by inhalation of diethyl ether until reached the respiratory rhythm regular blood taken 3 ml. Blood collected allowed to stand for 30 minutes at room temperature , then mess around at 2700 rpm for 10 minutes. Serum was separated, extracted and inserted into the bottle and then closed. the sample is then stored at a temperature of 4^0C. Furthermore, the determination of MDA levels in the blood of mice is measured by the amount of malondialdehyde reacts with the reagent acid tribarbiturat (mol/L) during the oxidative stress.

Laboratory Testing

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

a. Determination of MDA levels in rat blood is done using Mansila Espinosa. Measurement is based on the amount of malondialdehyde raects with acidic reagents tribarbiturat
b. Examination histopathological profile of pancreatic tissue 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 α = 0,05;
2. Homogeneity of variance were analyzed using Levene's test to determine whether variations in respective homogeneous group.
3. Analisis of differentiation on mean increase of MDA levels 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 $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characteristics of Euchresta horsfieldii lesch benn Leaf Extract

Euchresta horsfieldii lesch benn leaf extract condensed n-hexane maceration results give as much as 62.15% yield blackish green. While the results of antioxidant capacity against DPPH (*2,2-diphenyl-1-picrylhydrazyl*) showed that the seed extract has Euchresta horsfieldii lesch benn percentage reduction of 63.05% in 5 minutes and 82.90% within 60 minutes. Antioxidant capacity in the blood of rats was calculated based on the ability of catching free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) with the capture reaction.

The results of the separation of active compounds by GC-MS analysis showed that the Euchresta horsfieldii lesch benn leaf extract provides nine peaks with a retention time (tR), peak area (%), and has a different molecular weight. The compounds were detected in the n-hexane of Euchresta horsfieldii lesch benn leaf extract be presented in detail are presented in Table 1.

Table 1: The compounds were detected in the Euchresta horsfieldii lesch benn leaf extract

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	$C_{22}H_{42}O_4$	*Hexanedioic acid*
7	26.716	8.45	$C_{15}H_{24}N_2O$	*Matrine*
8	27.408	38.02	**BM = 302**	**New Compound**
9	27.891	2.30	$C_{24}H_{38}O_4$	*1,2-benzene dicarboxylic acid*

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

Decrease in blood MDA Wistar rats Diabetic

Data mean MDA blood hyperglycemia rats both pre and posttest are presented in Table 2 MDA profiles before and after treatment with various doses of Euchresta horsfieldii lesch benn leaf extract is presented in Figure 1.

Table 2. MDA Levels Before and After Treatment

Treatment	Observations MDA Levels (µmol/L)		
	Pretest The mean ± SD	Posttest The mean ± SD	Difference of MDA Levels (µmol/L)
Control group (K-) and (K+)	2,42 ± 0,13	0,61 ± 0,07	1,808 [b]
Euchresta L benn extract dose 0,5 mg	2,49 ± 0,12	0,96 ± 0,08	1,533 [d]
Euchresta L benn extract dose 2 mg	2,47 ± 0,09	0,72 ± 0,08	1,750 [c]
Euchresta L benn extract dose 5 mg	2,47 ± 0,09	0,46 ± 0,06	2,011 [a]

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-(Negative Control), K+(Positive Control/Glibenclamide)

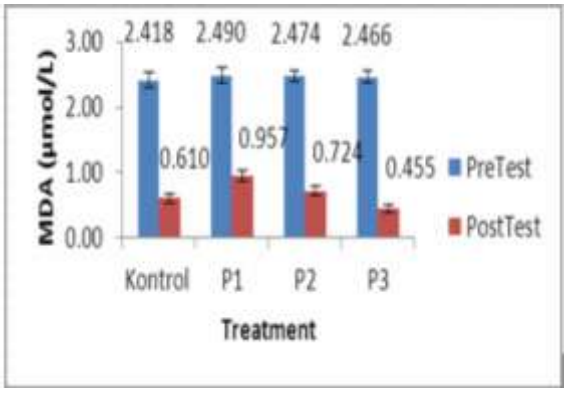

Figure 1: Profile of MDA Levels Before and After Treatment.

Test results with the Shapiro-Wilks normality and homogeneity test with Levene'stest shows that the data mean levels of MDA Wistar rats before and after administration of various Euchresta lesch benn 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 MDA 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 blood MDA 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.
Euchresta lesch benn extract with various doses can lower blood levels of MDA wistar rats diabetic. MDA mean data are shown in Table 5. Mean blood MDA levels of diabetic in rats negative control group (K-) and positive control group (K+) was 2.42 ± 0.13 µmol/L and 0.61 ± 0.07 µmol/L. From the same table can also be seen due to the extract MDA mean dose 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day in a row for the pre is (2.49 ± 0.12), (2.47 ± 0.09) and (2.47 ± 0.09) µmol/L, whereas for the posttest was (0.96 ± 0.08), (0.72 ± 0 , 08), and (0.46 ± 0.06) µmol/L. Compared with the positive control group (glibenclamide), the extract dose of 0.5 mg/kg bw/day decreased the levels of MDA, but not enough to dampen ROS produced by the metabolism of the body, so that ROS rapidly reacts with the double bond in the acid fatty acids to form lipid peroxidation is MDA on the cell membrane. The results are supported by Tjokroprawiro (2005), found that diabetic patients indicated a number of free radical formation such as H_2O_2, hydroxyl radicals that facilitate the formation of lipid peroxide (MDA) on the cell membrane. In the meantime, the results Wresdiyati *et al* (2003) states that α-tocopherol administration at a dose of 60 mg/kg/day for seven days can reduce the levels of MDA in rats with stress conditions.

The mean decrease in blood MDA levels were significantly diabetic rats already occurred in the extract dose of 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day with a value of p<0.05. Overall the data presented in Table 6. From the table it appears that the highest levels of MDA decreased blood wistar rats diabetic occurs in the extract dose of 5 mg/kg bw/day, which amounted to 0.50 µmol/L. This means that Euchresta lesch benn extract contains several classes of carboxylic acids such as Hexadecanoic acid, 9.12-hexadecadienoic acid, hexanedioic acid and 1,2-benzenedicarboxylic acid can lower blood levels of MDA in rats wistar due to lipid oxidation of unsaturated fatty acid chain length (unsaturated fatty acids). Declining levels of MDA in the blood may also prevent decreased membrane fluidity and cell damage. The results of animal studies indicate that consumption of saturated fatty acids cause a buildup chain unsaturated fatty acids in the body which can lead to increased toxic products of unsaturated fatty acid synthesis (lipid peroxidation), namely malondialdehyde that can cause damage to cell membranes. Kutlu et al (2009) stated that the granting of apricot kernel oil which is rich in fatty acids such as acids, oleic and linoleic acid, and the bioactive compounds such as thiamine. Riboflavin, vitamin C, α-tocopherol, sitosterol and kaempesterol able to reduce levels of MDA in male Wistar rats.

Profile of Histopathology Pancreatic β-cells 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 diabetic caused a dose 140 mg/kg bw can be seen in Figure 2,3,4, 5, 6 and 7.

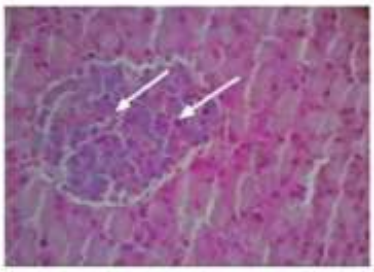

Figure 2: Normal Histopat Pancreas Wistar Rats (Magnification 400x)

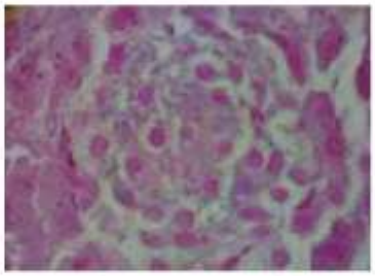

Figure 3: Histopat Pancreas Wistar Rats K- (alloxan) (Magnification 400x)

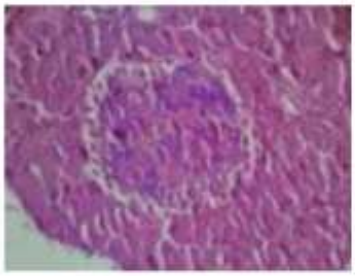

Figure 4: Histopat Pancreas Wistar Rats dose of 0.5 mg/kg bw (Magnification 400x)

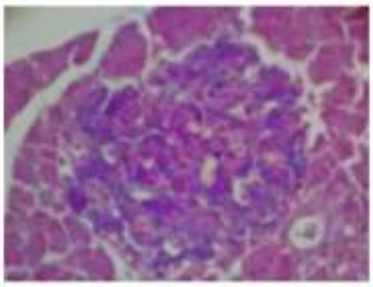

Figure 5: Histopat Pancreas Wistar Rats dose of 2 mg/kg bw (Magnification 400x)

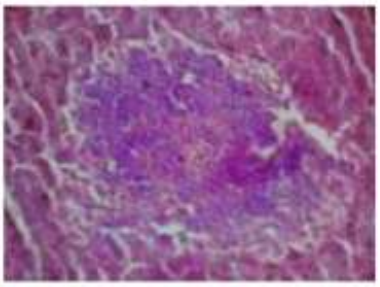

Figure 6: Histopat Pancreas Wistar Rats dose of 5 mg/kg bw (Magnification 400x)

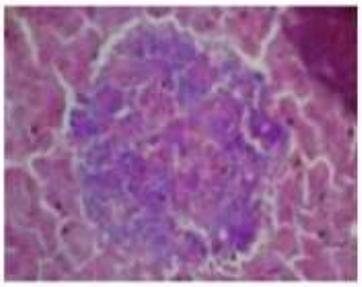

Figure 7: Histopat Pancreas wistar Rats K+ (Glibenclamide) (Magnification 400x)

Figure 2 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 3 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. Rats pancreatic β-cell degeneration to necrosis caused by alloxan induced a dose 140 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. Nowhere β-cells around the islets of Langerhans beta cells than in normal rats. Stroma reduced density of Langerhans on the island, there is edema, congestion, to undergo necrosis (cell death).

In Figure 4 above have been changes in pancreatic tissue morphology histopathology in rat islets of Langerhans due Euchresta horsfieldii lesch benn extract dose of 2 mg/kg bw compared with the negative control group(K-), although the amount of pancreatic β-cell degenerating to necrosis rather reduced. This means Euchresta lesch benn extract dose of 2 mg/kg bw can not help the process of pancreatic tissue repair damage caused by alloxan induced. In contrast to Figure 5 shows that employment Euchresta lesch benn extract dose of 5 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 Euchresta lesch benn extract dose of 5 mg/kg bw is likely Euchresta lesch benn extract dose of 5 mg/kg bw contains large insulincompare treatment groups in a dose of 2 mg/kg bw resulting in destruction of pancreatic β-cells quickly and normal. Figure nearing still visible above the cells undergoing necrosis.

Figure 6 does not appear on any cell degeneration to necrosis of the rat pancreatic tissue around the islands of Langerhans thus showing clear boundaries between β-

cells by α-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 7 morphological changes in the structure of the network in the rat pancreatic islands of Langerhans due antidiabetic drug administration (Glibenclamide) as a posttest control. No visible cytoplasmic granules and clear boundaries between β-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 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 400x magnification images of cells are shown in blue on the islets of Langerhans cell while the other is red.

CONCLUSION

1. The Euchresta horsfieldii Lesch benn leaf extract at a dose of 0.5 mg/kg bw/day, 2 mg/kg bw/day, and 5 mg/kg bw/day can prevent oxidative stress through decrease of malondialdehyde levels in diabetic rats
2. The Euchresta horsfieldii Lesch benn leaf extract at a dose of 5 mg / kg the best potential to prevent oxidative stress decreasing of malondialdehyde levels in diabetic rats. Decrease of malondialdehyde levels by (0,46 ± 0,06) µmol/L and differ statistically significant with $p<0.05$
3. The Euchresta horsfieldii lesch benn leaf extract improve the profile of histopathological tissue of wistar rat pancreatic β-cell damage caused by alloxan induced dose of 140 mg/kg bw.

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