POTENTIAL COMBINATION OF GEL AND ETHANOLIC EXTRACT SENGGANI LEAVES (Melastoma candidum D. Don) AGAINST TGF β1 ROLE IN HEALING OF SURGICAL WOUNDS ON THE MUCOSA PALATUM OF DMT2 MALE WHITE RATS

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Abstract

Data from the IDF (International Diabetes Federation) shows that Indonesia is in fifth position in the world and this number continues to increase every year, so glycemic control is needed in the form of drugs for this complication of diabetes mellitus. Treatment with herbal plants is safer than synthetic chemical drugs. One that is widely used is senggani plant. The researcher conducted an experimental research using The Post Test Only Control Group Design, 35 samples was randomly selected and divided into 7 groups. All groups were induced with Streptozotocin (STZ) 45 mg/kg BW to make the rats DMT2 and given High Fat Diet food and sucrose drinks. Group K- was not given any treatment, K+1 was given Metformin, K+2 was Metformin+Gengigel, P1 was only given Senggani leaf ethanol extract (EEDS) 500 mg, P2 (EEDS 500 mg + 5% EEDS gel), P2 (EEDS 500 mg + 10% EEDS gel) and P2 (500 mg EEDS + 15% EEDS gel). After 3 days of being induced by STZ, given treatment (Day 1) by first anesthetizing with ketamine 100mg/10ml, 20-40 mg/KG BW, a surgical wound was performed using a tissue punch with a diameter of 2.5 mm and a depth of 3 mm, Blood Glucose Levels, TGF β1 was measured. The normality and homogeneity results (Sig. TGF β1=0.219) were not significant on day 1 to day 16 because $p>0.05$, while the One Way Anova test was significant $p<0.05$, this indicated that there was an effect of treatment. The Post Hoc test in the form of the Tukey test showed that the P3 group was the most effective because EEDS contained tannins, saponins, flvonoids, phenols and glycosides, followed by K+2, while K- did not show much healing because there was no treatment for this group.

Keywords: Streptozotocin(STZ), Senggani Leaf Ethanol Extract (EEDS), Gel EEDS, Metformin HCL, Gengigel®, Blood Glucose Level, TGF β1.

Introduction

Type 2 DM treatment is compiled in international treatment guidelines such as the American Diabetes Association (ADA) as guidelines and recommendations in selecting therapy. There are various types of drugs, one of which is Metformin (ADA, 2021). Metformin (dimethylbiguanide) is the preferred first choice for oral blood glucose-lowering agents in managing type 2 diabetes (Bailey C.J., 2017), both in monotherapy and in combination therapy. Oral hypoglycemic drugs (OHO) such as sulfonylureas and metformin are noted to have good efficacy in controlling blood glucose in diabetes mellitus patients. Administration of metformin in patients with type 2 diabetes mellitus has rarely been reported to cause hypoglycemia (Gumantara M.P.B. et al., 2017), but has Side Effects of Drugs (ESO), namely gastrointestinal disorders such as diarrhea, nausea, vomiting, and flatulence (Sopianti D.S. et al., 2020).The structure and pharmacokinetics of Metformin are shown in Figure 1 (Bailey C.J., 2017).

Fig.1 The structure and pharmacokinetics of Metformin HCl (Bailey C.J., 2017).

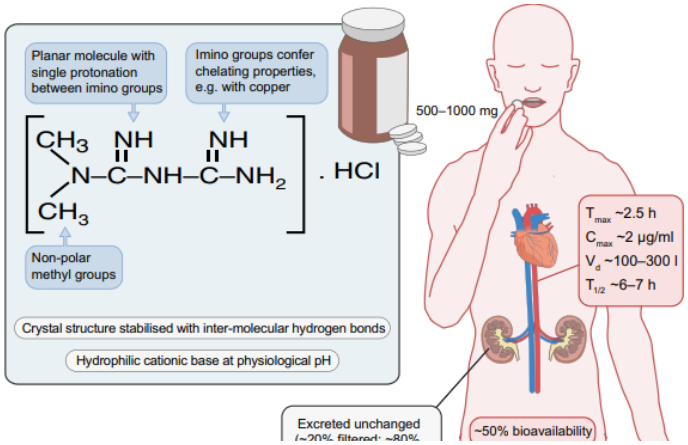
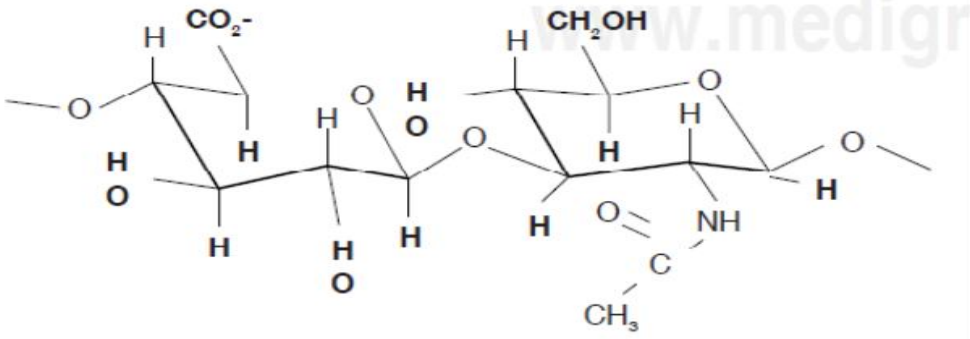


Fig.2 Structure of Hyaluronic Acid 0.2% (Gengigel® Gel)



Gengigel ® Gel Is a gel containing Hyaluronic Acid (HA isolated from eye fluids - bovine vitreous humor) is a glycosaminoglycan (a natural polysaccharide) that makes up connective tissue in various tissues, including skin and soft tissue - gum tissue in humans (Figure 2). HA with high Molecular Weight present in periodontal tissue is assisted to be synthesized by Enzyme Hyaluronan Synthase (HAS) (HAS1, HAS2 and HAS3) in various cells of periodontal tissue, including fibroblasts and keratinocytes in gingiva and periodontal ligament, cementoblasts in cementum and osteoblasts in alveolar bone HA which has a high molecular weight or the term High Molecular Weight Hyaluronic Acid (HMWHA) is considered to be able to optimally increase the stimulation of new tissue healing.

Indonesia, which consists of thousands of islands and is located in the tropics, so many herbal plants are found, one of which is often used is senggani (Melasoma candidum D.Don). The genus Melastoma has 22 species, 2 subspecies and 3 variants, all of which can be found in Southeast Asia (Halim S., 2022), including in Indonesia which is in the Southeast Asia region, can also be found in Madagascar, India to Australia. This plant very easy to find because it grows wild in places that get enough sunlight, such as on mountain slopes, shrubs, fields that are not too arid, or in tourist attraction areas as ornamental plants. Senggani in the form of shrubs or small trees as shown in Figure 3, can be found up to an altitude of 1,650 meters above sea level.

(Halim S., 2019).

Parts of the Melastoma plant such as leaves, roots, fruit, and seeds have various chemical constituents, especially in the leaves, which are very efficacious because they contain flavonoid compounds that function as anti-inflammatory, hypoallergenic, antioxidant and antibacterial and are compounds of phenols, which are phenols. is a strong antimicrobial element (Halim S., 2021), triterpenoids/steroids function as anti-inflammatories, saponins function as antiseptics, tannins function as astringents and glycosides as secondary metabolites (Halim S., 2022).

Fig.3 Senggani plant (Melastoma candidum D.Don) (Halim S., 2019)

MATERIALS AND METHODS

1. Apparatus and Instrumentation

1.1 Ethical Clearance (EC)

Prior to conducting research on experimental animals, Ethical Clearance was carried out at the Health Research Ethics Commission (KEPK) Universitas Prima Indonesia Registration Number at KEPPKN: 1271012S Registered/ Accredited Jl. Belanga No. 1 Simp.Ayahanda Medan.

1.2 Determination of Senggani Plants

The plant used has been identified at the Medanense Herbarium (MEDA), University of North Sumatra as a senggani leaf plant (Melastoma candidum D.Don).

1.3 Instrument

UV-Vis Spectrophotometer, Gravimeter, ELISA Reader, Glucodr Test Meter , CX33 Trinocular microscope with digital camera and Adobe Photoshop 7.

2. Materials

Streptozotocin (STZ), Ketamine, Metformin 500 mg, Gengigel® gel 20 ml, Senggani Leaf Ethanol Extract (EEDS) and EEDS gel 5%, 10% and 15%, Mouse Transforming Growth Factor β1 Elisa Kit Cat No E0660Mo, TGF β1 Mouse Monoclonal Antibody (10E5) BZ-089461F-AM.

3. Research Animal

Thirty-five adult male white rats of the Wistar strain (Rattus norvegicus) with inclusion criteria of body weight 150-200 grams, healthy and blood sugar levels > 200 mg/dl

4. Method development

4.1 Preparation of EDS Extracts and Gels

The extract was prepared by maceration using 80% ethanol because the polyphenols from senggani leaves are polar and relatively stable in acidic conditions so that the polyphenols in senggani leaves are more soluble in polar solvents such as methanol and ethanol. The process of making senggani leaf ethanol extract gel also uses a maceration technique. Starting from carbopol, it was developed with a portion of heated distilled water. Let stand in the mortar for 15 minutes, where the mortar was placed in a container filled with hot water to help the process of forming the consistency of the gel. The carbopol was added to glycerin, crushed, then added water and crushed again (mass 1). Mass 2 is formed from propylene glycol to which the rest of the water is added, then Nipagin is added, after which it is heated. Mix mass 1 with mass 2, then add Triethanolamine (TEA) drop by drop into the mixture, until a gel mass is formed. Stir until homogeneous and add the remaining distilled water to form a homogeneous gel mass. Add a small amount of the base gel to the mortar, then add the ethanol extract of senggani leaves and grind it until it is homogeneous. (Halim S., 2019).

4.2 Grouping of Research Samples

Rats were checked for blood glucose levels through the tail vein (Vena Cocygea). Rats were adapted for 1 week. All rats were marked first with a marker on the tail, then divided into 7 groups with 5 mice each, which included groups K-(no treatment), K+1 (Metformin), K+2 (Metformin+Gengigel®), P1 (500 mg EEDS), P2 (500 mg EEDS + 5% EEDS gel), P3 (500 mg EEDS + 10% EEDS gel), and P4 (500 mg EEDS + 15% EEDS gel). Before being induced with STZ, the rats were fasted for 12 hours, then their blood glucose levels were measured. The rat's blood glucose level should be within the normal range. STZ injection was given intraperitoneally and the dose was determined based on the rat's body weight. A single dose of STZ given 45 mg/kg BW (Saputra N.T., et al., 2018), left for 48 hours, while given HFD food and drinks mixed with sucrose solution.

4.3 In Vivo Research

Measurement of blood glucose levels in rats was carried out on the 3rd day after STZ injection and gave an increase in blood glucose response as an indication of experimental diabetes mellitus. Then the rats were anesthetized intra peritoneally (IP) using Ketamine HCl 100 mg / 1 ml in mice, at a dose of 20-40 mg / kg body weight of white male rats. First, apply 70% alcohol to the part of the rat to be anesthetized. Intraperitoneal injection is carried out by injecting it in the posterior 2/3 of the abdomen. Rats will fall asleep for approximately 3 minutes after injection and will regain consciousness for approximately 1 hour. This varies in each rat. After the rats fell asleep, surgery was then performed with a tissue punch with a diameter of 2.5 mm, with a depth of 3 mm, followed by medical therapy in the 6 groups except K-.

5. Collection of research samples

5.1 Phytochemical Tests on Senggani Leaf Ethanol Extract

Phytochemical screening procedures include tests for alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids / steroids and glycosides.

5.2 Blood Glucose Levels (BGL)

Examination of blood glucose levels was carried out before being induced by STZ, after 3 days after injection of STZ or 1st day after treatment, 8th and 16th day by enzymatic method, using the Gluko-Dr® tool to react specifically with glucose contained in blood. Glucose molecules that are oxidized by the Glucose Oxidase (GOD) enzyme produce electrons that are captured by the electrodes so that the glucose level is directly proportional to the electronic signal received. The amount of blood needed to measure blood glucose levels is 2.5-4.0 µL, the blood is placed on the right side of the test strip, the blood will be absorbed automatically and the measurement results will be read after eleven seconds on the Gluko-Dr test meter. Blood glucose levels are measured in units of mg/dL.

5.3 TGF β1 sample from blood serum

Blood collection from the retro orbital sinus of rats, Let the serum coagulate for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect supernatant without sediment. Use of this Enzyme-Linked Immunosorbent Assay (ELISA) sandwich kit for the accurate quantitative detection of mouse platelet-derived growth factor (also known as TGF). The results were read using a microplate reader with a wavelength filter of 450 + 10 mm.

5.4 Histopathological tissue samples of the palatal mucosa stained with IHK Anti TGF β1

Samples were taken from injured Wistar rats' palatal mucosa, then soaked in 10% formalin buffer for 2-4 hours, and followed by staining with Mouse Anti-TGF Beta 1 Antibody (10E5). Then observations were made with an Olympus CX 33 microscope assisted by a digital camera and software Adobe Photoshop CS 6.0 , the percentage of TGF expression was calculated using the ImmunoRatio software on five fields of view for each sample (Tuominen, et at., 2010).

Fig.4 Image analysis with ImmunoRatio software on five fields of view for each sample. http://www.ihcworld.com/ihc_scoring.htm (Tuominen, et at., 2010).

Quantitative Scoring Methods

1

Score	0	1+	2+	3+	4+
Positive Cells	<10%	10-25%	25-50%	50-75%	>75%

2

Score	0	1	2	3	4
Positive Cells	negative	few nuclei	10%	10-50%	>50%

3

Score	low level	high level			
Positive Cells	<20%	>20%			

4

Score	1	2	3		
Intensity of Staining	weak staining	moderate staining	strong staining		

5 Quick score (Q):
Results are scored by multiplying the percentage of positive cells (P) by the intensity (I).
Formula: Q = P x I; Maximum = 300

6. Statistical Data Analysis

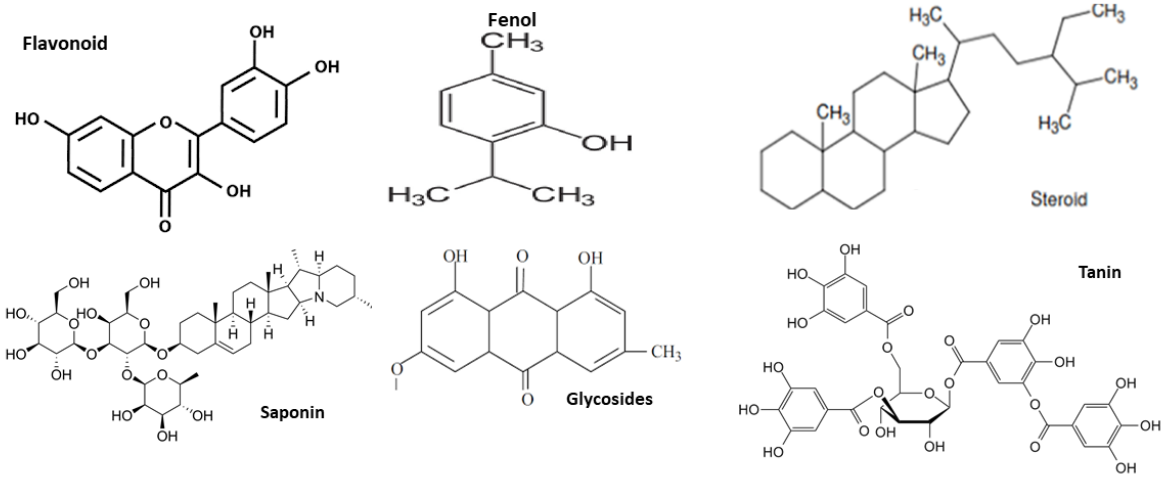
The data obtained is calculated average (Mean), SD (Standard Deviation) and Standard Error of Mean. The distribution of data was normal with the Shapiro-Wilk test and homogeneous with the Levine test, so the data were analyzed with the one way ANOVA test and continued with the Post Hoc Test, namely a comparison between groups using the Tukey's HSD test (honestly significant difference) and the SPSS (Statistical Product Services Solution) program.

RESEARCH RESULT

1. Results of Phytochemical Screening

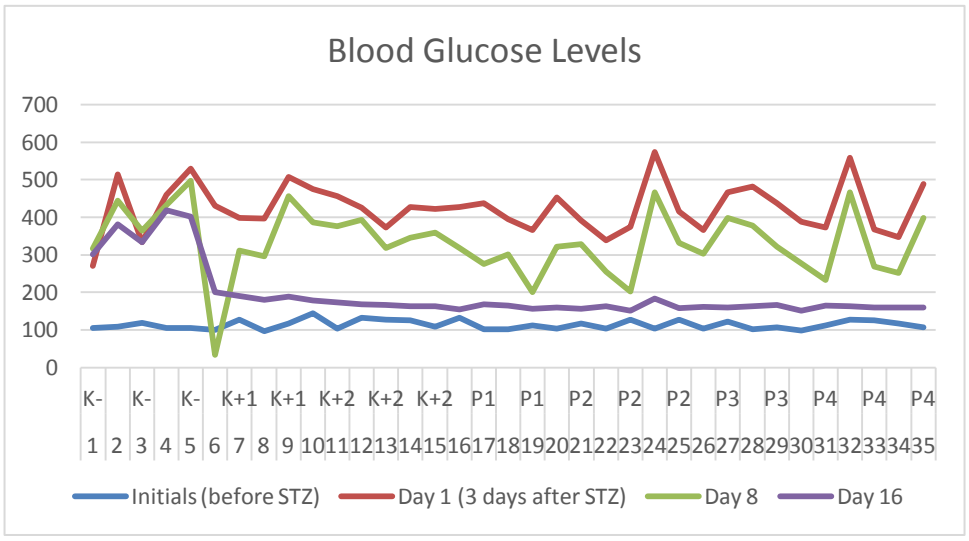
The results of the phytochemical screening of senggani leaf simplicia (Melastoma candidum D.Don) showed that in the senggani leaf simplicia contained tannins, the color of the simplicia liquid changed from green to blue or blackish green, saponins were seen above the senggani leaf simplicia in a test tube a steady foam appears for no less than 10 minutes as high as 1-10 cm. Flavonoids and phenols change the color of the simplicia in the test tube from green to orange-red and purple-red. Triterpenoids and steroids form a purple or brownish red ring on the cup indicating the presence of triterpenoids whereas if a greenish blue ring appears indicates the presence of steroids and glycosides a purple ring forms in the liquid in the test tube, while alkaloid compounds are not found in the senggani leaf simplicia. The chemical structure of the phytochemical composition of Melastoma candidum D.Don can be seen in Figure 5.

Fig 5 Phytochemical Consituent of Melastoma candidum D.Don



2. Blood Glucose Level (BGL)

Fig 6 Blood Glucose Levels of 35 Samples at baseline, day 1, 8 and 16.



In Figure 6 showed that the BGL of male white rats 3 days after being induced by STZ which is also the 1st day of treatment in the form of surgical wounds on the palatal mucosa of male white rats compared to the BGL in rats before STZ induced, there was an increase in KGD > 200 mg/day dL in all groups . The decrease in BGL began to occur after the 8th day until the last day of the study, namely on the 16th day in each group except K- whose blood glucose levels remained high because no treatment was given, only surgical procedures were given. This also shows a successful induction of STZ in male white rats so that these rats become Diabetes Mellitus Type 2 (DMT2).

Table 1. Mean Blood Glucose Levels (BGL) in Male White Rats Before and After STZ Induction

Report

Group		Initials (before STZ)	Day 1 (3 days after STZ)	Day 8	Day 16
K-	Mean	108.60	421.60	410.60	367.00
	N	5	5	5	5
	SD	6.066	114.476	70.992	48.306
	SE Mean	2.713	51.195	31.748	21.603
K+1	Mean	117.40	441.60	297.00	188.20
	N	5	5	5	5
	SD	19.957	48.475	160.519	8.786
	SE Mean	8.925	21.679	71.786	3.929
K+2	Mean	119.60	420.80	358.40	167.20
	N	5	5	5	5
	SD	13.240	30.655	28.962	4.324
	SE Mean	5.921	13.709	12.952	1.934
P1	Mean	110.00	416.00	283.60	161.00
	N	5	5	5	5
	SD	13.638	35.135	49.601	6.042
	SE Mean	6.099	15.713	22.182	2.702
P2	Mean	115.80	418.80	317.20	162.80
	N	5	5	5	5
	SD	12.518	91.196	99.452	12.071
	SE Mean	5.598	40.784	44.476	5.398
P3	Mean	106.60	428.20	335.60	160.60
	N	5	5	5	5
	SD	9.633	49.706	50.708	5.595
	SE Mean	4.308	22.229	22.677	2.502
P4	Mean	118.00	427.20	323.40	161.60
	N	5	5	5	5
	SD	8.972	92.216	103.283	2.702
	SE Mean	4.012	41.240	46.189	1.208
Total	Mean	113.71	424.89	332.26	195.49
	N	35	35	35	35
	SD	12.501	66.394	91.182	73.778
	SE Mean	2.113	11.223	15.412	12.471

N= Number

SD= Standard Deviation

SE Mean = Standard Error of Mean

In table 1 showed that the average BGL of male white rats experienced a very high increase (BGL > 200 mg/dL) after injection of STZ, but BGL decreased on the 8th to 16th day after being given treatment,

except for K - those who remained high in BGL, which only experienced a slight decrease, with an average difference on day 16 to day 1 after being induced by STZ of 54.6 mg/dL but this decrease varied in the other 6 groups, whereas difference - the average decrease in KGD on day 16 to day 1 (3 days after STZ) at K+1 was 253.4 mg/dL and K+2 was 253.6 mg/dL, so the decrease in KGD was not differed too much between the two groups.

In the treatment group, the average difference in BGL reduction from day 16 to day 1 was found to be highest at P3 of 267.6 mg/dL, P4 of 265.6 mg/dL, P2 of 256 mg/dL and P1 of 255mg/dL. The increase in BGL after STZ induced indicated that the male white rats had successfully developed type 2 DM because it was seen in the the STZ-induced K- group but there was no treatment, the KGD remained high from day 1 (3 days after STZ injection) to day 16 (Fig. 7). This is also in accordance with the results of a study by Kumar V., et al., 2013 which showed an increase in KGD in the 5 groups of white rats after being induced by STZ. Repeated administration of Melastoma malabathricum (MM) leaf extract orally to diabetic rats induced by STZ (streptozotocin) had an antidiabetic effect at different doses (100, 250 and 500 mg/kg) causing a significant decrease in blood glucose levels ($P < 0.001$), which shows that different doses of MM leaf extract give different effects. The maximum reduction rate of blood glucose was observed on day 28 (52.13%, 60.93% and 68.88% respectively), whereas glibenclamide showed 67.26% excursion blood glucose level compared to the diabetic control group MM 500 mg/ kg showed the maximum glucose lowering effect. Likewise, the results of a study by Balamurugan K., et al., 2014 in the 4 groups of alloxan-induced diabetic rats showed a significant increase in blood glucose levels ($P <0.01$) in group II (alloxan-induced but untreated group). compared to normal rats (Group I). The administration of M. malabathricum leaf extract 150 mg/kg BW and 300 mg/kg BW (Group III & IV) and 600 µg/kg BW glibenclamide (Group V) tended to bring significant parameters ($P<0.05$, $P<0$, 01) to normal.

Fig. 7 Comparison of Blood Glucose Levels on Day 6 to Day 1

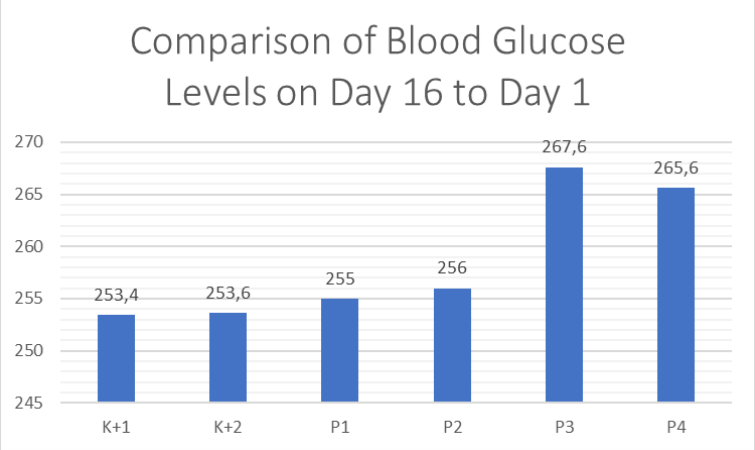
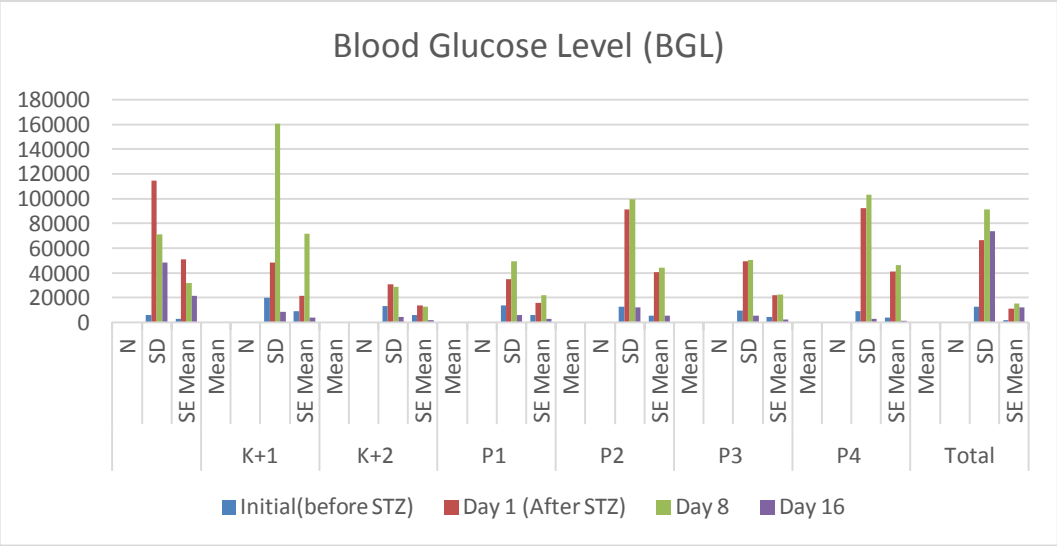


Fig.8 Potential of Melastoma candidum D.Don on Blood Glucose Levels of groups P1, P2, P3 and P4 compared to groups K+1, K+2 and K-, mean value + SEM, SD, N=5, P<0.05



The normality data for the average blood glucose levels of male white rats before and after being induced with STZ were tested using Shapiro Wilk because the number of data was less than 50 samples, the results were not significant because P> 0.05, so the data were normally distributed.

All sample data that were normally distributed were followed by a parametric inferential test with Levene's test on the homogeneity variant, and the results were also that all data were homogeneously distributed, with P> 0.05 (not significant).

The results of the One Way ANOVA test show the sig. < 0.05 (significant) then H0 fails to be accepted. Ha is accepted. It can be concluded that there is a difference in the mean BGL between the 7 groups. To find out which group makes a difference, the next test is the Post Hoc Test in the form of the Tukey HSD.

The results of the Post Hoc Test showed that in each group given treatment there was a decrease in BGL, especially on the 16th day, except for group K- with BGL > 200 mg / dL. The most effective group was the P3 group (500mg EEDS + 10% EEDS gel group) because it showed the highest value in the comparison of KGD reduction from the 16th day to the 1st day (Fig. 8).

3. TGF β1 Sample from Blood Serum

Fig. 9 Pre Test and Post Test TGF β1 Results by ELISA in DMT2 White Rats

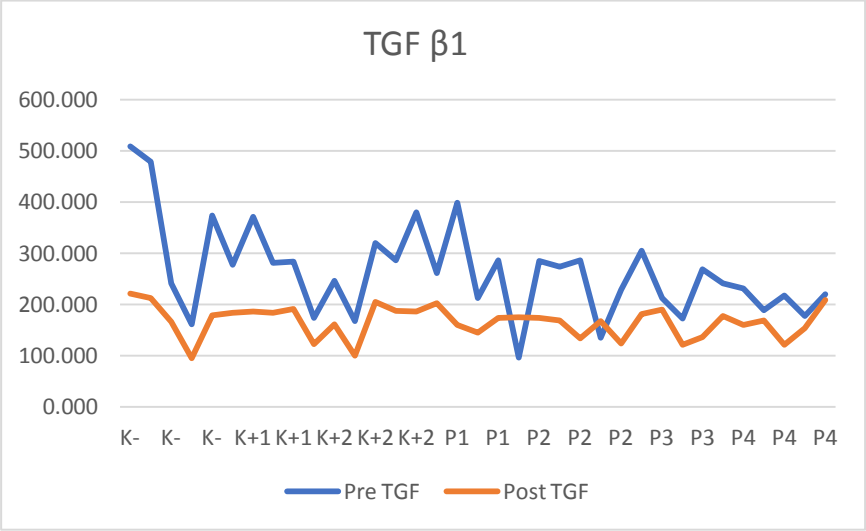


Figure 9 shows the results of the TGF β1 pre test by ELISA in white DMT2 mice in each group showing high levels of TGF β1, especially in the K- group which had very high TGF β1 levels compared to the other groups. This shows that there was a very high inflammatory reaction in the K-group after 3 days of being induced by STZ and on day 1 they were given surgical wound treatment on the palatal mucosa but no treatment was given. During the inflammatory phase, the broken blood vessels in the wound will cause bleeding and the body will try to stop it by means of vasoconstriction, retraction or shrinking of the broken blood vessels, and hemostatic reactions. Mast cells in the connective tissue produce serotonin and histamine which increase capillary permeability resulting in exudation, inflammation of inflammatory cells, accompanied by vasodilation which causes edema and swelling (Sjamsuhidajat, 2005). The inflammatory phase begins immediately after the trauma or injury and generally lasts for the 5th post-traumatic day. The main goals of this phase are generally hemostasis, loss of dead tissue and prevention of colonization and infection by pathogenic microbes (Gurtner, 2014). Injured blood vessels result in the mobilization of various blood elements to the wound site. Platelet aggregation will form a plaque in the injured blood vessels. During this process, platelets will degranulate and release several growth factors such as Platelets Derived Growth Factor (PDGF) and Transforming Growth Factor (TGF). The end result of the coagulation cascade of intrinsic and extrinsic pathways is the conversion of fibrinogen to fibrin (Gurtner, 2014).

The TGB β1 test results in Figure 9 also prove that the body has a natural ability to protect and restore itself. Increased blood flow to the damaged area, cleaning cells and foreign bodies, and early cellular development are parts of the healing process that occur normally without assistance, although some medicinal ingredients can support the healing process (Zahrok, 2009). This can be seen in the 6 groups that were given treatment, the results of TGB β1 levels in the post test decreased not so much, compared to the K- group which was not given treatment, there was a decrease in TGB β1 levels that were so much. The difference between the pre-test and the post-test in the TGB β1 test showed the best results were P4 followed by P3, P1, P2, K+1 and K+2.

Table 2. Average results (Mean) of pre-test and post-test TGF β1 by Elisa from the blood serum of male white rats induced by STZ and injured on the palatal mucosa

Report

		K-	K+1	K+2	P1	P2	P3	P4
Pre Test	Mean	353.226	277.566	279.808	250.936	242.202	240.072	207.207
	N	5	5	5	5	5	5	5
	SD	149.826	69.911	79.852	110.154	63.911	50.971	22.702
	SE Mean	67.004	31.265	35.711	49.262	28.582	22.795	10.152
Post Test	Mean	174.847	173.595	168.233	171.619	153.956	161.526	162.925
	N	5	5	5	5	5	5	5
	SD	50.442	28.781	40.754	21.595	22.785	30.344	31.216
	SE Mean	22.558	12.871	18.225	9.657	10.190	13.570	13.960
Total	Mean	264.037	225.581	224.021	211.277	198.079	200.799	185.066
	N	10	10	10	10	10	10	10
	SD	141.231	74.452	83.846	85.718	64.879	57.251	34.739
	SE Mean	44.661	23.544	26.514	27.106	20.516	18.104	10.985

N= Number
SD = Std. Deviation
SE Mean = Std. Error of Mean

Table 2 shows the results of the pre-test, the average TGF level in each group was relatively high due to a high inflammatory reaction (acute inflammatory phase) due to STZ induced and treatment in the form of surgical wounds in the palatal mucosa of rats. naturally to protect and restore oneself, especially in the oral cavity which is constantly moistened by the presence of saliva which has homeostatic functions of the teeth and mouth, antimicrobial and cleaning activity, degrades the cell walls of various bacteria and inhibits bacterial growth, neutralizes the production of acids which can damage teeth and repair network. (Benn A.M. and Thomson W.M., 2014).

The results of the post test mean TGF β1 levels showed a decrease in each group because the chronic inflammatory phase had been passed and had entered the maturation phase. The results of the post test mean TGF β1 levels in the K- group were still the highest even though there had been a decrease in TGF levels. the number of inflammatory cells was still high as a result the TGF levels remained higher compared to the other groups.

In the treatment group, especially the group with 500 mg EEDS and 15% (P4) and 10% (P3) EEDS gel, it showed that there was not much decrease in TGF levels in the post test. This also showed that the EEDS extract and EEDS gel, especially the P3 group and P4 is pro-inflammatory so that EEDS may be able to accelerate the inflammatory phase and also accelerate the wound healing process.

This is in accordance with the results of Revilla G.'s research in 2019 conducting an experimental study using 15 rats as research objects and divided into 3 groups namely the control group, the group given papain and the comparison group given silver sulfadiazine. Rats that suffered from burns were given treatment according to the group and on day 5 blood was taken through the eyes to obtain serum, then TGF-β levels were measured using the Elisa method. The results showed that the average TGF-β level in the control group was 317.72 pg/ml, the group given papain was 186.24 pg/ml and the control group was 192.11 pg/ml. This suggests that the papain enzyme is able to reduce TGF-β1 levels which are pro-inflammatory so that papain may be able to accelerate the inflammatory phase and also accelerate the wound healing process.

In Figure 10 the difference between the Post Test and Pre Test TGF-β1 levels by Elisa shows that the K- group shows the highest difference with a very large decrease in TGF-β1 levels in the post test and is followed by groups with K+2, K+1, P2 , P1, P3 and P4.

Fig. 10 Difference between Post Test and Pre Test TGF β1 Levels

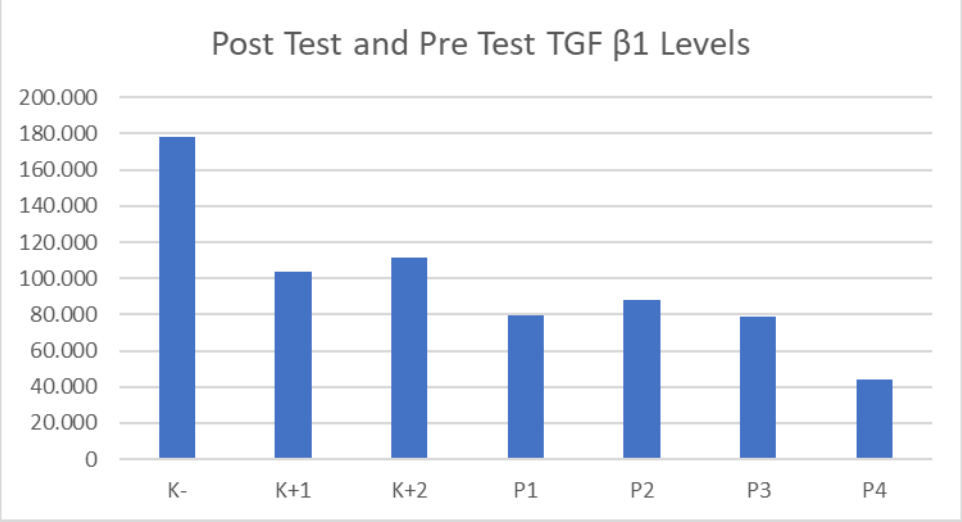
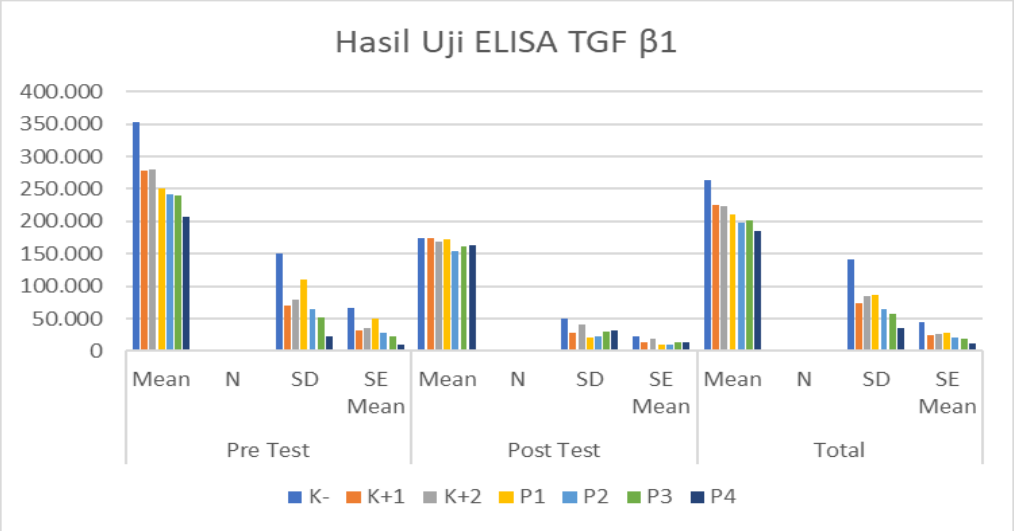


Fig. 11 Potential of Melastoma candidum D.Don on TGF β1 levels from groups P1, P2, P3 and P4 compared to groups K+1, K+2 and K-, mean value + SEM, N=5, P<0.05.



The normality data for the average TGF β1 levels from the blood serum of male white rats showed insignificant results because P>0.05, so the data were normally distributed. All sample data that were normally distributed were followed by a parametric inferential test with Levene's test on the homogeneity variant, and the results were also that all data were homogeneously distributed, with P> 0.05 (not significant).

The results of the One Way ANOVA test show the sig. <0.05 (significant) indicates the potential for the combination of EEDS gel and extract on surgical wound healing in the palates of male white rats. It can be concluded that there is a difference in the mean TGF β1 level between the 7 groups. To find out which group makes a difference, the next test is the Post Hoc Test in the form of the Tukey HSD.

The results of the Post Hoc Test showed that in each group that was given treatment there was a decrease in KGD in the post test, except for the K- group with TGF β1 levels which were still quite high compared to the other groups, even though they had entered the maturation phase. The most effective group was the P4 group (500 mg EEDS + 15% EEDS gel), followed by the P3 group (500 mg EDS + 10% EDS gel) because it showed the fastest healing phase compared to the other groups (Figure 11).

4. Histopathological Test of TGF β1 from Samples of Palatal Mucosal Tissue with IHK Anti TGF β1 staining

Fig. 12 Expression of anti TGF β1 in palatal tissue 100 x ,

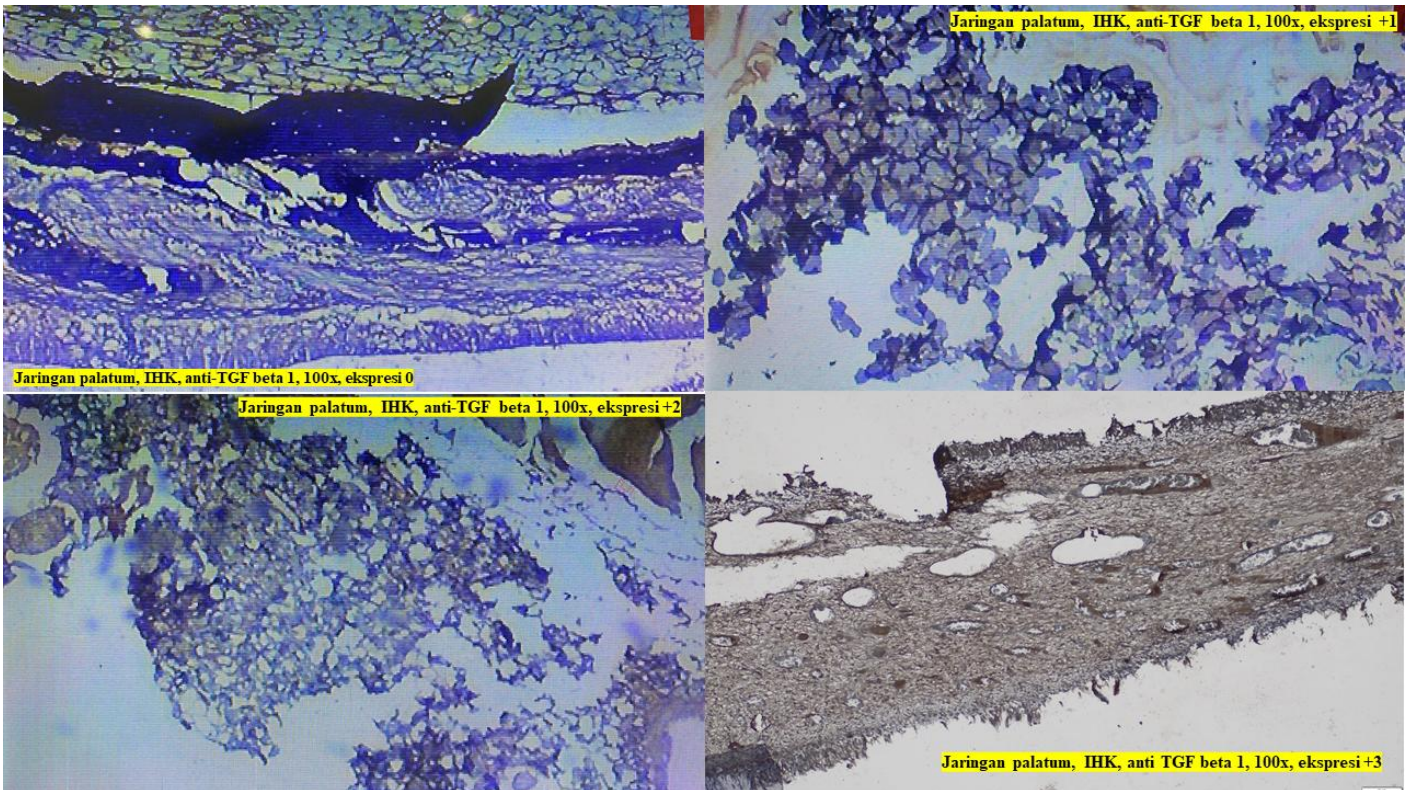


Fig. 13. TGF β1 intensity in surgical wounds in the palates of white DMT2 male rats per 5 field of view with IHK staining in the 7 groups studied.

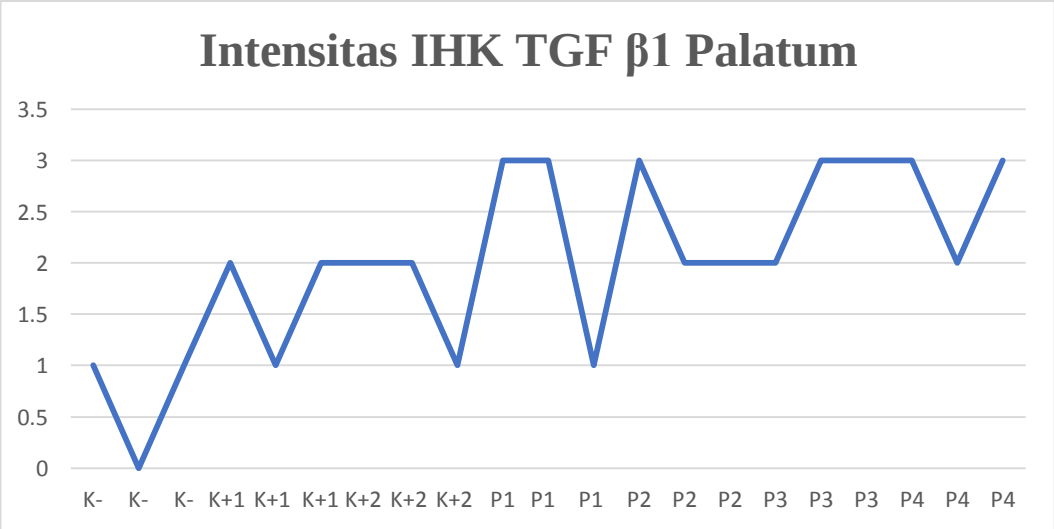


Figure 13 shows that the highest expression of TGF β1 was in the P3 and P4 groups followed by P1 and P2, K+2 and K+1 and the lowest was in the K- group, because EEDS contains Flavonoids that can stimulate macrophages thereby increasing the production of growth factors such as Transforming Growth Factor (TGF).

Tabel 3. Results Average (Mean) Expression of TGF β1 in the palate given surgical wounds per 5 field of view DMT2 male white rats with IHK staining

Report

Intensitas (I) +

Kelompok	Mean	N	Std. Deviation	Std. Error of Mean
K-	.67	3	.577	.333
K+1	1.67	3	.577	.333
K+2	1.67	3	.577	.333
P1	2.33	3	1.155	.667
P2	2.33	3	.577	.333
P3	2.67	3	.577	.333
P4	2.67	3	.577	.333
Total	2.00	21	.894	.195

Table 3 shows the average (Mean) expression of TGF β1 in the palate that was given a surgical wound per 5 fields of view of DMT2 male white rats with CPI staining showing the highest average (Mean) expression in groups P3 and P4, followed by P2 and P1, as well as K+2 and K+1 , while K- showed the lowest TGF expression.

Normality test results for TGF β1 expression on the palate treated with surgical wounds per 5 fields of view of male white DMT2 rats with CPI staining showed insignificant results with P>0.05 so the data were normally distributed.

Table 4. Homogeneity test of TGF β1 expression in the palate treated with surgical wounds stained with IHK per 5 field of view of male white DMT2 rats

Test of Homogeneity of Variances

Intensitas (I) +

Levene Statistic	df1	df2	Sig.
1.600	6	14	.219

In Table 4, the homogeneity test for TGF β1 expression in the palate that was given a surgical wound per 5 field of view of DMT2 male white rats with IHK staining showed insignificant results with H0 being accepted and Ha being rejected. The data is homogeneously distributed, so the test is continued with One Way Anova.

Table 5. One Way Anova Test for TGF β1 expression in the palate treated with surgical

wounds stained with IHK per 5 field of view in male white DMT2 rats

ANOVA

Intensitas (I) +

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.333	6	1.556	3.267	.032
Within Groups	6.667	14	.476		
Total	16.000	20			

Table 5 shows the One Way Anova Test for TGF β1 expression in the palate treated with surgical wounds stained with IHK per 5 fields of view of male white rats with DMT2 showing significant results (P< 0.05), thus showing that the group given therapy proved effective in reducing ROS in rats Male White.

Result and Discussion

DISCUSSION

In this study, to produce type 2 diabetes mellitus (DMT2) in male white rats, they were induced with a single dose of STZ 45 mg/kg body weight. Three days after being induced with STZ, blood glucose levels in each group became high (> 200 mg/dL). This STZ induction action succeeded in making white rats become DMT2, this can be seen from the K-group whose blood glucose levels remained high until the end of the study because no treatment was given. STZ penetrates Langerhans β cells via the GLUT 2 glucose transporter and results in DNA changes to pancreatic β cells. DNA by STZ via the nitrosourea group results in damage to pancreatic β cells (Saputra N.T., et al., 2018). This damage causes an increase in free radicals in the body causing an imbalance between oxidants and antioxidants. This condition leads to oxidative stress (Gusbakti R., 2022).

Blood Glucose levels in the 6 groups that were given medication therapy showed a decrease in KGD, with the highest decrease in the P3 group (500mg EEDS + 10% EEDS gel) and the lowest in the K- group (the group that was only induced by STZ and was given surgical wound treatment in palatal mucosa but not given treatment). The process of reducing KGD in the P3 group occurred because EEDs contained Flavonoids which function as anti-inflammatories, antioxidants and stimulate the induction of Vascular Endothelial Cell Growth Factor (VEGF) which has a function to facilitate growth factors (Izzati, U. Z., 2015), Tannins function as astringents (Habibie A,. 2020), Saponins have the ability as cleansers and antiseptics (Ramadhani R. et al., 2017), Triterpenoids / Steroids as anti-inflammatories (Halim S., 2022) and glycosides which are secondary metabolites.

The results of the ELISA test on TGF β1 from the blood serum of male white rats showed that the K-group was relatively high because there was a high inflammatory reaction due to STZ-induced and given treatment without treatment, followed by the STZ-induced group and given treatment and treatment, namely the K + 2 group, K+1, P1, P2, P3 and P4. The results of the post test mean TGF levels in the K-group remained the highest even though there had been a decrease in TGF levels due to the inflammatory phase having been passed and entering the maturation phase. the mouth is always moist

by the presence of saliva (Benn A.M. and Thomson W.M., 2014). TGF-β plays a role in increasing fibroblasts, cell chemotaxis, modulating collagen, collagenase expression, which in turn produces matrix-producing cells for the rapid deposition of new connective tissue in the wound area during the proliferation phase followed by the maturation phase (Diegelmann and Evans, 2004) . Among all TGF-β isoforms, TGF-β1 plays an important role in the differentiation of protomiofibroblasts into mature myofibroblasts during the remodeling phase (Chitturi et al., 2014; Gilbert et al., 2016; Van Beurden et al., 2005).

Histopathological test of TGF β1 expression from Palatal Mucosal Tissue Samples with IHK Anti TGF β1 staining was calculated by using the Immuno Ratio software in five fields of view for each sample (Tuominen, et at., 2010) showed the results of Average (Mean) Expression The highest TGF β1 was in the P3 and P4 groups, followed by P2 and P1, and K+2 and K+1 , while K- showed the lowest TGF expression. This shows that the P3 and P4 groups have the potential to accelerate the healing phase by reducing inflammatory reactions through the phytochemical compounds contained in them, namely Flavonoids containing quercetin and quercitrin which have anti-inflammatory effects, Phenols, triterpenoids/steroids, saponins, tannins and glycosides. The results of histopathological tests showed that the combination of 500 mg EEDS and 10% EEDS gel was the most effective, although the combination of 500 mg EEDS and 15% EEDS gel showed the same results as the P3 group, but with a concentration of 10% it already gave the same results as 15%, so the P3 group determined as the most effective group.

Conclusion:

Based on the results of the research that has been done, it can be concluded that

a. The class of chemical compounds found in simplicia and Senggani leaf extract are saponins, flavonoids/phenols, glycosides, tannins and terpenoids/steroids, which function as anti-inflammatory and antioxidants.

b. Combination extract and gel of senggani leaf ethanol extract (Melastoma candidum D. Don) has an effect on wound healing in male white rats (Rattus norvegicus) with the most effective concentration being 10%.

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