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EFFECT OF MIXED GRASS JELLY (*MESONA PALUSTRIS* BL) AND OTHER INGREDIENTS EFFERVESCENT POWDER IN DIABETIC RATS

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***Abstract*— The present study aimed to test the effect of grass jelly (*Mesona palustris* BL) and other ingredients effervescent powder (EV) on the blood glucose level and histology of pancreas in rats with diabetes mellitus induced by alloxan (DMIA rats). DMIA rats were orally administered with effervescent powder at dose 126 mg/ 200 g body weight (bw) (EV1), 252 mg/200 g bw (EV2) and 378 mg/200 g bw (EV3) for 4 weeks. The result showed after administration of EV to the DMIA rats, the blood glucose levels was significantly decrease than the positive controls. The statistical data indicated that EV3 was not significant different (α=0.01) to those administered glibenclamide at dose 0,135 mg/200 g bw. Histologically, damaged islets was observed in DMIA rats but was less damaged in treated DMIA rats. These findings showed that an effervescent powder of *Mesona palustris* BL may prevent development of increased blood glucose level and enhance improvement of damaged in diabetic pancreas.**

***Key words*— Blood Glucose, Effervescent, Histophatology, Mesona palustris BL.**

I. INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to glucose overproduction or hyperglycemia [1]. The number of diabetic patients will reach 366 million in 2030 [2]. At present, the treatment of DM mainly involves sustained hyperglycemia reduction by use of chemical drugs. However, due to unwanted side effects, the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of DM.

The oral dosage forms are the most popular way of taking medication despite having some disadvantages like slow absorption and thus onset of action is prolong. This can be overcome by administrating the drug in liquid from but, many APIs have limited level of stability in liquid form. So, Effervescent acts as an alternative dosage form. Effervescent is a powder intended to be dissolved or dispersed in water before administration. In addition to active ingredients, it generally contains mixture of acids/acid salts and carbonate and hydrogen carbonates which release carbon dioxide when mixed with water [3].

Recently, there have been growing interests in the application of natural components as antidiabetic agents [4]. Ones of the material that can be used as a functional food are grass jelly (*Mesona palustris* BL.), pandan (*Pandanus amaryllifolius*) leaves and red ginger (*Zingiber officinale* Linn). The grass jelly is consumed as both an herbal drink and a jelly-type dessert in China [5]. Pandan, are often used to give a refreshing, fragrant flavor and a pleasant aroma, mainly due to the presence of 2-acetyl-1-pyrroline [6]. The genus Zingiber comprises about 85 species of herbs mostly distributed in East Asia and tropical Australia. Many of these are used as food and for traditional treatment of a variety of ailments [7].

The *Mesona palustris* BL has been shown as antimutagenic, to treat hypertension and diabetes [5, 8-10]. Sheu et al [10] isolated hypoglycemic substance, ursolic acid, and several other compounds, including oleanolic acid, β-sitosterol, stigmasterol, β-sitosterol glycoside, α-and β-amyrin, and maslinic acid from grass jelly. It was reported that phenolic acids could be the important antioxidant components in grass jelly [11]. The objectives of this study were to test the effect of grass jelly (*Mesona palustris* BL) and other ingredients effervescent powder (EV) on the blood glucose level and histology of pancreas in rats with diabetes mellitus induced alloxan (DMIA rats).

II. MATERIALS AND METHODS

A. Materials and Chemicals

Dried grass jelly (*Mesona palustris* BL) was purchased from a farmer in Magetan, East Java-Indonesia. Fresh pandan leaves (*Pandanus amaryllifolius*) and red ginger (*Zingiber officinale* Linn) were purchased from farmer in Malang, East Java-Indonesia. Plant materials were obtained in August 2013. Tartaric acid, sodium bicarbonate, citric acid, stevia, and PVP. Analytical grade of ethanol, dextrin, Na2CO3 were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Folin-Ciocalteu's reagent was purchased from Merck (Darmstadt, Hesse, Germany). DPPH (1,1'-diphenyl-2-picrylhydrazyl) radical and other chemicals were purchased from Sigma Chemicals (St. Louis, Missouri, USA). Glucose GOD FS was purchased from DiaSys (Holzheim, Germany).

B. Materials and Chemicals

The dried grass jelly was cut into small pieces and ground into a fine powder. The pandan leaves and red ginger were cut into small pieces. A grass jelly sample and pandan leaves (1:1) were extracted with 20-times volume of 100° C boiling water for 2 h. The extracts were add 5% (w/v) of dextrin then dried by vacuum dryer (WIKA, Germany) (65oC, 7 h) into a powder form. The red ginger also extracted with 20-times volume of 100° C boiling water for 2 h. The extracts were add 5% (w/v) of dextrin then dried by vacuum dryer (WIKA, Germany) (65oC, 7 h) into a powder form.

The powder of grass jelly and pandan (45%): red ginger (18%): citrate acid (12%): tartrate acid (6%): stevia (5%): PVP (1%) were mixed to made acid granulation. The obtained mass passed through sieve no.20#. In base granulation, natrium bicarbonate (12%): PVP (1%) were mixed and passed through

sieve no.20#. Finally, acid granules and base granules were mixed to made effervescent powder [12].

C. Hypoglycemic Activity of Effervescent

The animal procedures carried out in this study were approved by Animal Care and Use Committee of Brawijaya University, Malang, Indonesia. Twenty-four Male *Rattus norvegicus* of 2-3 months of age (weighing 200-300 gram) were purchased from Gadjah Mada University, Yogyakarta, Indonesia. The rats were randomly divided into six different treatment groups of four animals each. The rats were housed individually in stainless steel cages and subjected to 12-h light/dark cycle. All rats were provided with AIN-93M and water ad libitum. The treatment groups were treated orally by gavage with effervescent powder dissolved in aquades for 4 weeks. The experimental groups were classified as follows:

1. Normal rats with aquades (negative control) (NK)
2. DMIA rats with aquades (positive control) (PK)
3. DMIA rats with effervescent powder doses 126 mg/200 g body weight (bw) (EV1)
4. DMIA rats with effervescent powder doses 252 mg/200 g body weight (bw) (EV2)
5. DMIA rats with effervescent powder doses 378 mg/200 g body weight (bw) (EV3)
6. DMIA rats with glibenclamide doses 0.135 mg/200 g body weight (bw) (GB)

In order to induce diabetes, DMIA rats were given intraperitoneally 80 mg/kg of alloxan. Blood glucose levels in rats were measured using Glucose GOD-POD methods activity [13]. Mix 10 µl serum or standard with 1000 µl reagent, incubate 20 minutes at 20-25°C. Read absorbance against the blank within 60 min at wavelength 500 nm. At the end day of treatment, animals were anesthetized with lethal dose of chloroform. Then the pancreas sample were collected and each organ was fixed in 10% neutral phosphate-buffered formalin. Sample then dehydrated in graduated ethanol (50-100%), cleared in xylene and embedded in paraffin. The hepatic sections (4-5 µm) were examined with a photomicroscope (40x) after staining with haematoxylin and eosin (h-E) dye. The histopathological studies were carried out at Anatomy Pathological Laboratory, Malang, Indonesia.

D. Evaluation of Effervescent

EV were studied for its water content, total phenol and IC_{50} values using different phytochemical test [14–16]. EV also studied for its compressibility index, angle of repose and solution time [17–22].

E. Statistical Analysis

The data obtained is expressed as mean ±SD. The data was analysed by analysis of variance (ANOVA) followed by post hoc tests (Least Significance Different and Duncan's Multiple Range Tests (DMRT)) (α = 5% for effervescent and α = 1% for in vivo) using Microsoft excel software.

III. RESULTS

A. Evaluation of the Effervescent

The data on mean water content, total phenol, IC_{50}, compressibility index, angle of repose and solution time of the effervescent are given in Table 1.

TABLE I. EVALUATION OF THE EFFERVESCENT

Parameter	Result
Antioxidant activity IC_{50} (ppm)	63.33±2.32
Total phenol (ppm)	64.92±5.65
Water content (%)	3.37±0.01
Angle of repose (θ)	29.5±0.55
Compressibility index (%)	21.33±2.66
Solution time (second)	9.1±0.42

Values are expressed as mean ± SD (n=6)

B. Effect of Effervescent on Blood Glucose Level (BGL)

The data on mean fast BGL in rats are showed in Table 2. Compared with the level at week 0, blood glucose level (BGL) of rats administered with effervescent showed a decrease at week 1st, 2nd, 3rd, and 4th. Animals treated with EV1 caused 22.80% reduction in the BGL during the first week followed a reduction of 25.56%, 33.09%, and 42.87% in the 2nd, 3rd and 4th week, respectively. Animals treated with EV2 also showed hypoglycaemic activity, caused 18.20%, 23.48%, 36.71%, and 46.87% reduction in the BGL during the 1st, 2nd, 3rd and 4th week, respectively. The hypoglycaemic activity also showed by EV3 that caused 20.29%, 30.09%, 40.53% and 50.30% reduction in the BGL during the 1st, 2nd, 3rd and 4th week, respectively The effervescent at dose 378 mg/200 g bw showed better hypoglycemic activity than others effervescent used in this research, the blood glucose decreased from an initial level of 212,68 mg/dl to 105.70 mg/dl after 28 days. Table 2 showed rats administered with effervescent at dose 378mg/200 g bw was not significant different to those administered glibenclamide at dose 0,135 mg/200 g bw.

Treatments	Week 0 (mg/dl)	Week 1 (mg/dl)	Week 2 (mg/dl)	Week 3 (mg/dl)	Week 4 (mg/dl)	Percentage of decreasing blood glucose (%)
Control rats	75.78±0.70	76.79±1.02	79.26±1.04	79.84±0.97	80.51±0.82	-6.24 ±1.39
DMIA rats (positive control)	218.31±9.97[a]	222.38±8.62[a]	229.09±5.37[a]	228.31±5.76[a]	230.72±5.27[a]	-5.68 ±2.52[a]
DMIA rats with effervescent (125mg/200 g body weight)	220.42±7.90	170.17±5.31[b]	164.08±4.87[b]	147.49±4.67[b]	125.93±4.67[b]	42.87 ±3.37[b]
IA rats with effervescent (252mg/200 g body weight)	216.43±14.92	177.04±11.69[b]	165.61±11.69[b]	136.99±1.54[b]	115.00±1.28[b]	46.87 ± 3.76[b]
DMIA rats with effervescent (378 mg/200 g body weight)	212.68±8.37	169.53±4.67[b]	148.69±4.52[b]	126.48±4.20[b]	105.70±4.62[b]	50.30 ±1.89[b]
Glibenclamide	210.00±11.01[b]	196.03±3.01[b]	136.06±6.36[b]	110.02±4.75[b]	95.08±2.98[b]	54.72 ±3.07[b]

TABLE II. BLOOD GLUCOSE LEVEL IN RATS AT WEEKS 0,1,2,3, AND 4

Values are expressed as mean ± SD (n=4)
aSignificantly different (α= 0,01) from control
bSignificantly different (α= 0,01) from DMIA rats

C. Effect of Effervescent on Histological of Rats Pancreas

Histological examinations of the rat pancreas sections under study are presented in Figure 1-6. Administration of alloxan decreased the number of β-cells and the sections from DMIA rats demonstrated shrunken islets of Langerhans with degenerative necrosis (Fig.2). In the sections from effervescent treated rats, islets of Langerhans appeared less shrunken compared to those from the untreated group (Fig 3-5).

IV. DISCUSSION

The present study evaluated the anti-hyperglycemic activity of effervescent powder made from *Mesona palustris* BL, pandan leaves and red ginger extracted by aquades in alloxan induced diabetic rats. Alloxan-induced diabetic rats have been widely used as a model for evaluation of antidiabetic activity [23-25]. The cytotoxic action of this diabetogenic agents is mediated by reactive oxygen species (ROS). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals formed by Fenton reaction with massive increase in cytosolic calcium concentration, causing rapid destruction of β-cell [26]. The destruction of β-cell showed in Figure 2.

At the beginning of the study, the average values of BGL among the five groups, except control group were in the range of 212-220 mg/dl. According to the WHO (1999), subjects in this study had high values of BGL, which therefore disposed them to Diabetes Mellitus (DM). Although the mechanism of the blood glucose-lowering of *Mesona palustris* BL is unknown, the hypoglycemic effects of the effervescent may be due to phenol inside *Mesona palustris* BL [11]. Phenol is the antioxidant which protect cell against oxidative stress. Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defense capacity of the body, is closely associated with number of DM. Oxidative stress possibly causes various forms of tissue damage in patients with diabetes such as pancreatic β-cell dysfunction in type 2 diabetes. The antioxidant treatment probably exerts its effect in association with the presence of hyperglycemia; for example by protecting β-cells from the toxic effect of ROS produced under hyperglycemic conditions [27-28].

Results obtained in the present study showed that histology of rat pancreas from DMIA rats with effervescent nearly normal pancreatic architecture and an islet with normal appearance. Administration of effervescent to diabetic rats improved the histological of pancreas. The mechanisms of these effervescent actions were unknown, but it may be due to its antioxidant-activity, such as total phenol.

V. CONCLUSSION

The effect of grass jelly (*Mesona palustris* BL) and other ingredients effervescent powder (EV) on the blood glucose level and histology of pancreas in rats with diabetes mellitus induced by alloxan were investigated, and a superior anti-hyperglycemic effect on the blood glucose was observed in EV3. These findings suggest grass jelly (*Mesona palustris* BL) and other ingredients effervescent powder has potential as a treatment to lower blood glucose level, with mechanism awaiting further investigation.

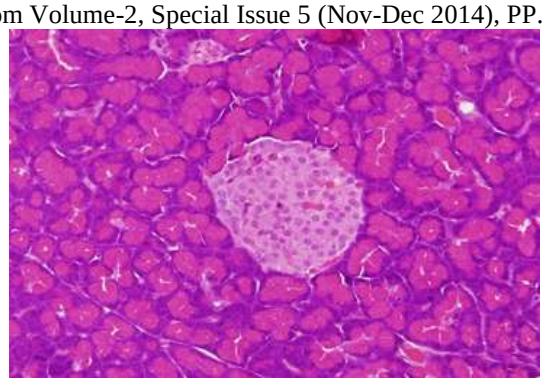

Fig. 1. **A histology of rat pancreas from the control group showing the closely packed pancreatic acini somposed of cicle shaped cells (40x)**

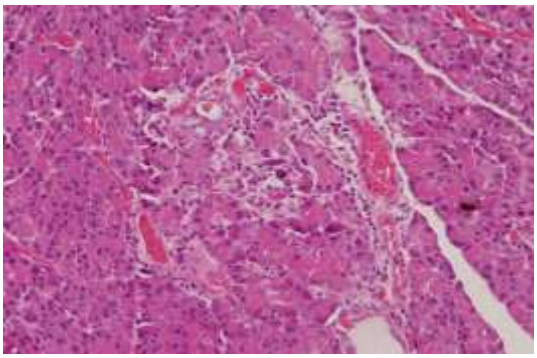

Fig. 2. **A histology of rat pancreas from DMIA rats showing loss of cell integrity and damaged islets (40x)**

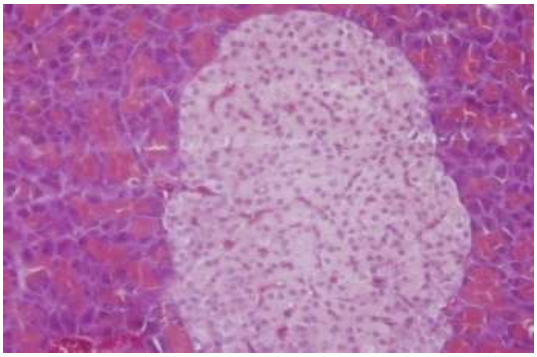

Fig. 3. **A histology of rat pancreas from DMIA rats with effervescent doses 125 mg/200 g bw (40x)**

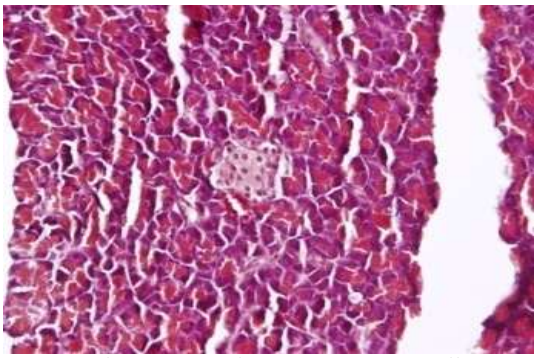

Fig. 4. **histology of rat pancreas from DMIA rats with effervescent doses 252 mg/200 g bw (40x)**

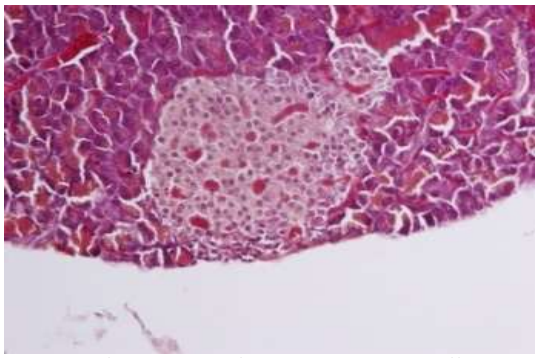

Fig. 5. **histology of rat pancreas from DMIA rats with effervescent doses 378 mg/200 g bw (40x)**

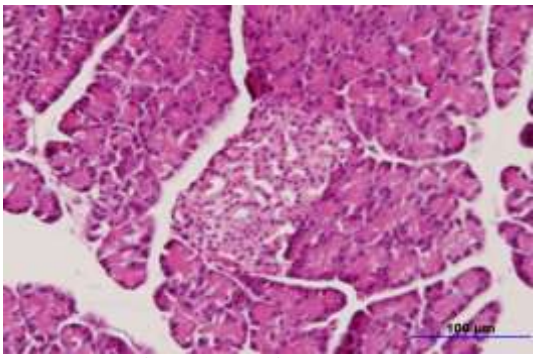

Fig. 6. **A histology of rat pancreas from DMIA rats dosed with 0.135 mg/200 g bw glibenclamide (40x)**

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