Antidiabetic Effect of the Combination of Garlic Peel Extract \textit{(Allium sativum)} and Onion Peel \textit{(Allium cepa)} in Rats with Oral-Glucose Tolerance Method

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ABSTRACT:
Diabetes mellitus threat in the middle of the society has become a scare disease because in each 10 seconds in the world people die because the complication of that disease. The application of natural drug has been applied empirically by the society, such as the application of the extract of garlic peel and onion peel each has been reported has hypoglycemic effect and potential as an antioxidant which can repair damage cell body, include pancreas. The aim of this study is to understand the effect of the combination of garlic peel extract and onion peel extract to the decreasing of blood glucose level in normal rats compare to the combination of metformin and glibenclamide and to the decreasing of blood glucose level in rats induces diabetic drug compare to pen insulin. This laboratory experimental study aims to understand the anti-diabetic effect of the combination of garlic \textit{(Allium sativum)} peel extract and onion \textit{(Allium cepa)} peel extract in rats induced glucose per orally and alloxan. Test of anti-diabetic effect, test animals are divided into 7 group, 3 groups to oral glucose tolerance test in normal rats (negative control, positive control, and extract combination) and 4 groups to test in rats induced diabetes (negative control, positive control, diabetic control, and combination extract). The result of oral glucose tolerance test to the normal groups reveal that the combination of this extract significantly decrease blood glucose level compare to the negative control group specifically in the 0, 30\textsuperscript{th}, 60\textsuperscript{th}, and 90\textsuperscript{th} minutes (p<0.05). In the test to the diabetic induces rats reveal that the combination of this extract also significantly decrease the blood glucose level compare to diabetic group (p<0.05), meanwhile the result is not real difference to the positive control group (p>0.05). This study shows that the combination of peel garlic extract and onion peel extract have the ability to decrease the blood glucose level both in normal rats induced glucose and rats induced diabetic.

KEYWORDS: garlic peel, \textit{Allium sativum}, onion peel, \textit{Allium cepa}, Diabetes Mellitus, oral glucose tolerance.

INTRODUCTION:
Diabetes mellitus threat in the middle of the society has become a scare disease because in each 10 seconds in the world people die because the complication of that disease. Diabetes Mellitus is a chronic disease occurring when body unable to produce enough insulin or when the body do not utilize insulin effectively then cause metabolic disease characterized by chronic hyperglycemic as well as abnormal metabolism of carbohydrate, protein and fat\textsuperscript{1}.

One of the goal therapies of the Diabetes Mellitus patient is to control blood glucose by giving oral hypoglycemic
or insulin. Meanwhile, the side effects are undeniable. This reason has attracted the researchers to apply natural sources as one of the alternative in curing diabetes mellitus. In Indonesia, there are various research that apply natural resources and use in the society. But the plant waste is rarely apply, such as the waste of garlic and onion produced from household waste.

In the previous research stated that onion peel extract contains flavonoid compound which potential as an antioxidant and prevent free radical as well as repair damage cell, include pancreas². Another research conducted by Setiawan et al. (2011) conclude that alicin and allii content in garlic has hypoglycemic effect which is more effective than glibenclamide³.

Those are becoming reasons for the researchers to conduct the study about the affectivity of the combination of garlic peel extract and onion peel extract compare to the hypoglycemic potency of the combination of glibenclamid and metformin in white wistar rats with glucose tolerance method, thus this study can be a new drug entity to cure type 2 Diabetes Mellitus.

**METHODS:**

**Plant Materials:**
Garlic peel (*Allium sativum*) and onion peel (*Allium cepa*) collected from Unaaha district, Konawe regency, Southeast Sulawesi

**Plant determination:**
Sample determination conducted in Biology Faculty of Haluoleo University

**Plant Processing:**
Garlic peel (*Allium sativum*) and Onion peel (*Allium cepa*) were separated from the bulb then cleaned and dried. Sample dried by the air for 2-3 days, then powdered.

**Plant Extraction:**
Garlic peel powder (*Allium sativum*) and onion peel powder (*Allium cepa*) each extracted with maceration method using 96% ethanol solution with comparison sample and solution 1:4. Maceration process conducted for 5 days while stirring some times. Each pulp then remacerated until the solution is clear. Each maceration results collected then evaporated using rotary evaporator.

**Anti-diabetic Assay:**

**Tested Animal Conditioning:**
Test animal applied in this research is male wistar rats aged 8 weeks, weight 150 -200 g and caged separated as test group. Rats are adapted in cage for a week before treated. Cage condition stated in 28-32 °C and dark-light cycle for 12 hours each. Rats were feed standard diet food and water *ad libitum*⁴,⁵. Volume given is 2 ml based on normal rat gastric volume that is 3-5 ml⁶.

**Anti-diabetic Effect Test:**
In this test, test animal divided into 7 groups, 3 groups for oral glucose tolerance test in normal rats and 4 groups for induced-diabetic rats. Each group consists of 5 rats.

**Glucose Tolerance test in normal rats:**
Rats divided into 3 groups, consist of:
- Group I: Negative control (Na CMC)
- Group II: Positive control (Glibenclamid + Metformin)
- Group III: Treated Group; Combination of ethanol extract of garlic peel and onion peel (800 mg/kg BW and 200 mg/kg BW per orally)

On the first day before treatment all groups are fasting for 20 hours. After 20 hours, blood glucose were measured from each groups. Then, each group induced glucose orally, after that the blood glucose levels are measured in the 30th minute. After that, they were treated as each group need. Performed blood glucose measurement in the 0, 30th, 60th, 90th and 120th minute.

**Test in Diabetic- Induced rats:**
- Group I: Negative control (Na CMC)
- Group II: Positive control (Insulin Pen)
- Group III: Diabetic control
- Group IV: Treated Group; Combination of ethanol extract of garlic peel and onion peel (800 mg/kg BW and 200 mg/kg BW per orally)

On the first day before treated all rats are fasting for 20 hours, then blood glucose levels were measured. Diabetic induction in rats conducted by injecting aloxan monohydrate (150mg/kgBW) intra-peritonially. Blood glucose level of rats then measured in the 2nd day, 24 hour after injecting aloxan. Rats stated as diabetic when they have 200-260 mg/100 ml of blood glucose level. But that does not occur in this study because of several reasons such as fasting blood glucose level which is low or stress condition that may be faced by the rats⁵,⁷.

Next, rats were treated based on the treatment groups. On the 3rd day, all treatment groups were measured their blood glucose level. Treatment continue until day 15. In the 15th day, performed blood glucose level at the 0, 6th, 12th, 18th and 24th hour.

**Data Analysis:**
Study result stated in average±SEM. Data significance analyzed by *One-way Analysis of Variance* (ANOVA) (program SPSS 16.0) with *post hoc* LSD’s *test*. Data is significant if P values is less than 0.05.
RESULT:
The Result of Anti-diabetic Assay Combination of Garlic Peel Extract (Allium sativum) And Onion Peel (Allium cepa) In Rats with Oral-Glucose Tolerance Method.

![Graph showing glucose levels](image)

* KGDP : Blood Glucose Level during fasting (mg/dL), KGDG : Blood Glucose Level After induced glucose (mg/dL), 0 : Blood Glucose Level after treatment at 0 minute (mg/dL), 30 : Blood Glucose Level after treatment at 30 minute (mg/dL), 60: Blood Glucose Level after treatment at 60 minute (mg/dL), 90 : Blood Glucose Level after treatment at 90 minute (mg/dL), 120 : Blood Glucose Level after treatment at 120 minute (mg/dL)

Fig 1: The result of Glucose Tolerance Test in Normal Rats

![Graph showing glucose levels](image)

*KGDP : Blood Glucose Level during (mg/dL), KGDA : Blood Glucose Level 24 hours after inducing alloxan (mg/dL), H+3 : Blood Glucose Level H+3 treatment (mg/dL), H+15(0) : Blood Glucose Level H+15 Treatment hour 0 (mg/dL), H+15(6) : Blood Glucose Level H+15 Treatment hour 6 (mg/dL), H+15(12): Blood Glucose Level H+15 Treatment hour 12 (mg/dL), H+15(18) : Blood Glucose Level H+15 Treatment hour 18 (mg/dL), H+15(24) : Blood Glucose Level H+15 Treatment hour 24 (mg/dL)

Fig 2 : The test result in rats induced diabetic

DISCUSSION:
Normality test performed to each treatment group after induced glucose and treatment at the 0, 30th, 60th, 90th and 120th minute using Shapiro-Wilk test reveals that at the 0, 30th, 60th, 90th and 120th treatment data normally distributed with significant value 0.156, 0.209, 0.375, and 0.442 (p>0.05). Meanwhile, in blood glucose level after inducing glucose and treatment at the 120th minute data distributed abnormally with significant score each 0.02 and 0.35 (p<0.05).

The result of the analysis show that there is a real difference between extract treatment group and negative control group after treatment at 0, 30th, 60th, 90th and 120th minutes with significant score 0.005, 0.002, 0.050 and 0.042 (p<0.05) each. The blood glucose level is decreasing after given the extract. In positive control group if compare to negative control group show no significant difference after treatment at the 0, 30th, 60th, 90th and 120th minutes with significant score each 0.351; 0.533, 1.000, 0.624 and 0.107 (p<0.05). A real significant difference between extract and positive control after the 0, 30th, and 60th minutes with significant score each 0.013; 0.009 and 0.006(p<0.05).

That study, similar with previous research which state that compounds in garlic peel acts as anti-diabetic. Compounds contain in garlic peels such as: SPC (S-propil-sistein), SEC(S-etyl-sistein), SMC (S-metil-sistein) include organosulphur group, garlic also contain organoselenium and tellurium, flavonoid bioactive compound such as kaemferol-3-O-b-D-glukopirosa, Iso-ikamnetin-3-O-b-D-glukopirosa, crucial fructopeptide compounds such as Na- (1-deoy-D-fructose-1-yl)-L-arginin, protein with beta-carotene, Thiamine (Vit.B1), Riboflavin (Vit.B2), Niacin (Vi.B3), Pantothenic acid (B5), alicin and alliin, Vitamin C, Calsium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc, and chemical compound that contain in onion bulb that is quersetine, a flavonoid compound act as antioxidant, revitalize damage beta pancreas cell thus can produce insulin or can be functioned in certain mechanism to fasten blood glucose reuptake.

In the treated group which induced alloxan, conducted normality test using Shapiro-Wilk reveal that data distribute normally on H+15 treatment the 0, 6th, 12th, 18th and 24th hour with significant score each: 0.064, 0.771, 0.735, 0.608 and 0.777 (p>0.05). For H+3 treatment data distributed abnormally with significant P value as much as 0.024 (p<0.05).

Based on the data analysis conducted shows significant real difference on H+3 treatment in control group positive and extract treatment compare to diabetic group with significant value each 0.012 and 0.002 (p<0.05). But in positive control compare to treatment with extract reveal no significant difference with significant value is 0.649 (p>0.05).

From data analysis on H+15 treatment at 0 hour, shows significant difference in positive control and extract treatment compare to diabetic group with significant
score 0.006 and 0.000 (p<0.05). In positive control group compare to extract treatment show no real significant difference with significant score 0.069 (p>0.05).

Treatment on H+15 the 6th hour based on analysis result reveal real significant difference between diabetic group and extract treatment with significant score 0.020 (p<0.05). Analysis result in positive control group compare to diabetic group and extract treatment show no real significant difference with significant score 0.075 and 0.999 (p>0.05)

Treatment on H+15 the 12th hour based on analysis result reveal real significant difference in diabetic group if compared to positive control group and extract treatment with significant score 0.004 and 0.001 (p<0.05). But unreal significant difference showed in the comparison between positive control group and extract treatment with significant score 0.580 (p>0.05).

Data analysis on H+15 treatment at 18th hour, shows significant difference in diabetic group when compare to diabetic group and extract treatment with significant score 0.001 (p<0.05). There is no real significant difference between positive control group and extract treatment with significant score 0.984 (p>0.05)

At 24th hour of H+15 treatment shows significant difference in diabetic group when compare to control positive group and extract treatment with significant score consecutively 0.007 and 0.001 (p<0.05). The comparison between positive control group and extract treatment reveal unreal significant difference, with significant score of 0.095 (p>0.05)

CONCLUSION:
Based on the result of research after analyzing statistically and discussing, we conclude that combination of ethanol extract of onion peel (800 mg/kg BW and 200 mg/kg BW per orally) able to increase tolerance to glucose thus effective to the decrease of blood glucose level in normal rat compared to the combination of metformin and glibenclamid and combination of ethanol extract of garlic peel and onion peel (800 mg/kg BW and 200 mg/kg BW per orally) able to decrease blood glucose level in rat induced diabetic, but the result is no real difference with positive control group induced insulin.

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