HYPOGLYCEMIC EFFECT OF *ZINGIBER ZERUMBET* ETHANOLIC EXTRACTS AND *CHANNA STRIATA* POWDER IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

The ethanolic extracts of the *Zingiber zerumbet* rhizome contains flavonoid and zerumbon compounds, the fish meat powder from *Channa striata* has an albumin content. The active compounds was contained in both materials had activity that can lower blood sugar levels in diabetic rats. This study aims to determine the hypoglycemic effect of each ingredients and the combination of *Z. zerumbet* rhizome extract and *C. striata* powder in alloxan-induced diabetic rats. This research uses pre and post test method with the control group design. The treatment was consisted of 5 groups, the first group was given aquades (as negative control), the second group was given glibenclamide with dose 5 mg/kgBW (as positive control), the third group was given 300 kg/kgBW of *Z. zerumbet* rhizome extract, the fourth group was given of *C. striata* fish powder 300 mg/kgBW, and the last group was given a combination materials with doses respectively, 300 mg/kgBW. The ingredient was given for 12 days and blood was taken on days 3, 6, and 12. The results shows the percentage of decrease in blood sugar of combination materials of 70.4% is greater than the single content of *Z. zerumbet* and *C. striata*, with the value of successive activity of 62.9% and 34.4%, respectively. The antidiabetic activity of combinations of materials had produced not different significant ($p > 0.05$) compared with single of *C. striata* powder as well as the *Z. zerumbet* rhizome extract.

Keywords: ***Hypoglycemic Effect,*** *Zingiber zerumbet*, *Channa striata,* ***Alloxan-induced Diabetic Rats***

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INTRODUCTION

Diabetes mellitus is a metabolic disorder disease with decreased insulin production or insulin resistance characterized by decreased blood sugar levels (Dipiro *et al*., 2008). The incidence of diabetes mellitus will increase from 2000-2030 with an incidence rate of 2.8% to 4.4% or about 171 million people (Wild *et al*., 2004). The incidence of diabetes in Asian countries is ranked the greatest, nearly 80% of diabetics live in low-and middle-income countries (Akter *et al*., 2014).

In Indonesia, cork fish or *Channa striata* has albumin content that has various properties one of them as antidiabetic. Giving 0.14846 mL/day of cork fish powder (*C. striata*) was able to lower blood sugar and regenerate the pancreas Langerhans island by 68.78% in mice induced by alloxan (Aisyatussoffi and Abdulgani, 2013).

For many years *Z. zerumbet* or *Zingiber zerumbet* are widely used as traditional medicine (Joseph *et al*., 2015). *Lempuyang Emprit* belonging to the family of *Z. zerumbet* has been studied to have anti-diabetic activity (Sakika *et al*., 2014). *Z. zerumbet* ethanolic extracts were reported to have activity of lowering blood sugar levels in diabetic nephropathy rats induced with streptozotocine dose of 300 mg/kg (Tzeng *et al*., 2013). Based on the toxicity test, *Zingiber zerumbet* extract has a safe kaempherol compound used for antidiabetic therapy (Nag *et al*., 2013). In addition, zerumbone compounds in *Zingiber zerumbet* extract can lower blood sugar levels in mice (Koga *et al*., 2016).

Based on the research that has been done above, cork fish and *Zingiber zerumbet* have effect on the decrease of blood sugar level. The combination of *Z. zerumbet* and cork fish that has never been done previously. Hence, the purpose of this study is to determine the effect of anti-diabetic combination of cork fish and *Zingiber zerumbet* when compared with the effect of single extract of *Zingiber zerumbet* and cork fish extract.

MATERIALS AND METHODS

Tools and Materials

Tool: stainless steel vessel, vacuum pump, beaker glass, eppendorf tube, analytical balance, porcelains bowls, CAS scale, spuit syringe 3,0 mL, skinner knife, waterbath (Memmert), vacuum rotary evaporator (IKA RV 10), Buchner funnel, vortex, sentrifuge minispin (eppendorf), vet, white tip, blue tip, micro pipettes, spectrophotometer UV-VIS (star Dust FC*15), waterbath, horn spoon , glassware pyrex.

Material: cork fish powder (*Channa striata*) produced by CV Jadiid Herbal Solo, simplicia *Zingiber serumbet* rhizome, technical ethanol 96%, aquades, rattus novergicus galur wistar from Rumah Tiput farm Klaten, reagen kit *Glucose* PAP SL (ELITech Clinical System), aloksan (Sigma Aldrich), technical ethanol 70%.

Zingiber zerumbet Extraction

Simplisian *Zingiber zerumbet* rhizome 3 kg is soaked in 6L ethanol 96%, well stirred and covered through for four days and stirred at schedule. The result of the soak is filtered by using vaccum buchner. Filtrat formed is evaporated by means of vacuum rotary evaporator with 60^{o}C that thick extract is formed.

Alloxan Pre-eliminary Test

Alloxan dose is made 3 doses series 125 mg/kgBB, 150 mg/kgBB, and 200 mg/kgBB. Alloxan is injected intraperitoneally and the level later be measured in the fourth day. 150 mg/kgBB can increase the glucose level of the rat.

Preliminary Test of Zingiber zerumbet Extract and Cork Fish Powder

The rats are divided into two groups, each group consist of 3 rats. The given dose treatment of the cork fish powder and *Zingiber zerumbet* extract is 150 mg/kgBB and 300 mg/kgBB, the sugar level of the rats then be measured. 300 mg/kgBB is used as it can decrease the level of sugar in the blood.

Extract Treatment and Powder to the test Animal

Sugar level of rats with diabetes after allloxan induction is measured and compared with the level baseline. The treatment group are divided into 5 groups, each groups consist of 3 rats. Having been:

Given extract of all level for 7 days.

The treatment group are as follows:

Group I : negative control given aquades p.o

Group II : positive control given glibenklamid 5 mg/kgBB

Group III : given *Zingiber zerumbet* rhizome extract 300 mg/kgBB

Group IV : given cork fish extract 300 mg/kgBB

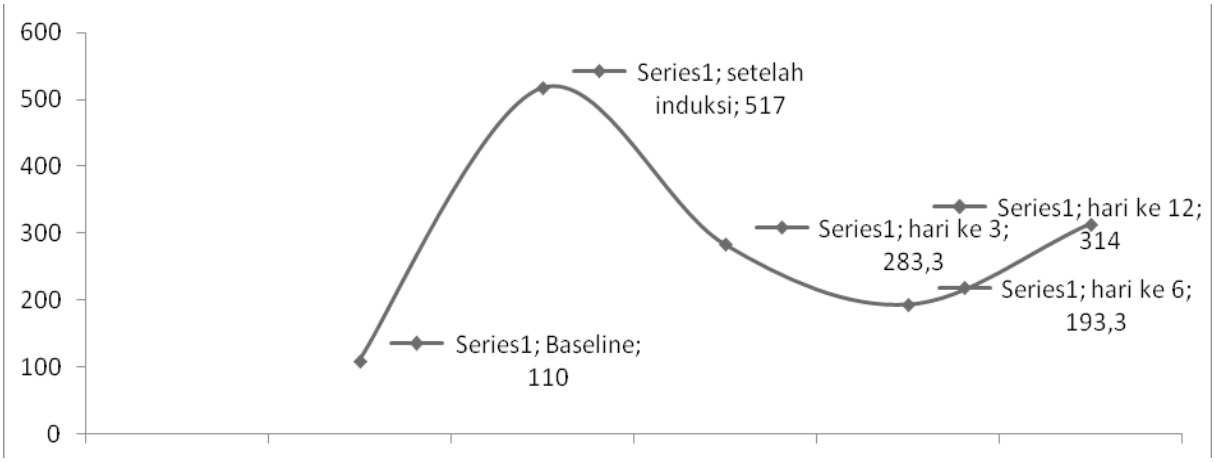
Group V : given cork fish extract combination: *Zingiber zerumbet* extract (1:1)

Measuring blood sugar level.

Blood is taken from vein lateralis of the rats tails 0,5 mL then centrifugated for 10 minutes at 13.400 rpm speed. After centrifuge process, serum taken is used as sample to measure the level 5mL Supernatant taken and set in to the vet and added with 500 mL reagen kit *Glucose* PAP SL. The sample is read by using spektrophotometer Visible *Start Dust FC 15 DiaSys.*

RESULTS & DISCUSSION

Extraction process of *Zingiber zerumbet* rhizome by using maceration method. This maceration method is generally known to be easier and cheaper. The maceration process using 96% ethanol solvent can dissolve better chemical compounds especially flavonoid compounds (Agustiningsih *et al*., 2010). From the process of maceration *Zingiber zerumbet* as much as 3 Kg soaked in 96% ethanol as much as 6L obtained extract of *Z. zerumbet* as much as 98,3 gram. Diabetes mellitus is a degenerative disease that can be caused by degenerative stress (Ullah *et al*., 2016). Oxidative stress can be interpreted in the presence of an imbalance between pro-oxidants and antioxidants (Ullah *et al*., 2016). Pro-oxidants that cause oxidative stress are ROS (Reactive Oxygen Species) (Ullah *et al*., 2016). The mice were induced by alloxan doses of 150 mg and their blood sugar levels were observed for 4 days to determine the stability of blood sugar level increase in mice. Alloxan is a diabetogenic agent with a sulfidril protein-binding mechanism (-SH) on the glucose bond side of glucokinase which then forms a disulphide bond and enzyme inactivation (Rohilla and Ali, 2012). The bond between the alloxan and thiol can cause the formation of dialuric acid which then reduces the metal ions and forms the oxidation product (Rohilla and Ali, 2012).



Noted:
Group 1: baseline
Group 2: after induction
Group 3: day 3
Group 4: day 6
Group 5: day 12

Figure 1 The graph of increase sugar level of rats before and after induction

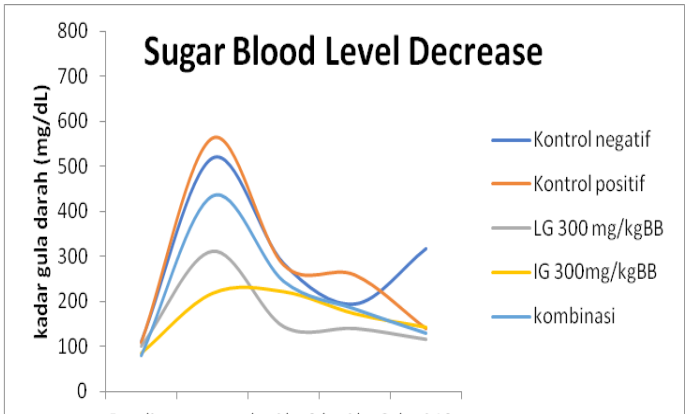
Based on the graph above, rats glucose level of the negative control group having been given alloxan 150 mg/KgBB dose shows there is an increase up to the day 12 (last day). The treatment injected alloxan to rats with diabetes each given extract and extract combination.

Table 1 The result of rats blood sugar level before test and after treatment on day 3, 6, and 12.

Group	Baseline (mg/dL)	Blood glucose level (pre test) (mg/dL)	Post test
			Day 12 Treatment (mg/dL)
Negative Control	113	676	382
	111	375	252
	106	500	314
X ± SD	110,0±3,6	517,0±151,2	316,0±65,0
Positive Control	102	483	167
	108	605	90
	111	599	158
X ± SD	107,0±4,6	562,3±68,8	138,3±42,1
Z. zerumbet	101	231	60
dose 300 mg/kgBB	100	212	121
	100	493	166
X ± SD	100,3±0,9	312,0±157,0	115,7±53,2
Cork Fish	85	216	87
dose 300 mg/KgBB	70	202	189
	100	233	151
X ± SD	85,0±15,0	217,0±15,5	142,3±51,5

Exctract Combination	84	391	180
Z. zerumbet and Cork fish	58	203	46
	94	710	160
X ± SD	78,7±18,6	434,7±256,3	128,7±72,3

The data above shows that rats given *Zingiber zerumbet* extract dose 300 mg/kgBB up to the day 12 (last day) has decrease sugar level 115,7±53,2 mg/dL. The rats given 300 mg/kgBB fish extract can decrease the sugar level 142,3±51,5 mg/dL. The extract combination given *Zingiber zerumbet* extract and cork fish extract each dose 300 mg/kgBB , rats sugar level decrease up to 128,7±72,3 mg/dL.



Noted: 1 = baseline; 2 = after induction; 3 = day 3; 4 = day 6; 5 = day 12

Fig. 3. Sugar Blood Level Decrease

The graph 4 shows there is a decrease of sugar level on day 3. The decrease reach normal level 144 mg/dL shown by *Z. zerumbet* dose 300 mg/kgBB. On day 6 all the given extracts and powders show the decrease of sugar level to the normal level <200 mg/dL. On day 12, all extracts and positive control shows normal sugar level. Hence, the result suggest the same sugar level. However, ELG shows the best decrease sugar level 115,7 mg/dL smaller than positive control 138,3 mg/dL. Obi *et al.*, (2015) claims that glibenclamide has decrease activity of sugar level on day 5. while Happy *et al.*, (2014) states that it takes 14 days to reach normal sugar level.

ANOVA test on day 12 shows statistically significant decrease (P<0,05) for and cork fish powder compare with negative control. Nevertheless, there is no statistical significant difference if compare to positive control fish extract. Similarly, ELG-SIG combination shows no statistical significant difference compare to positive control and single treatment.

Z. zerumbet has cell contributes in the process of rats sugar level decrease called zerumbon (Koga *et al.*, 2016). Zerumbon in *Z. zerumbet* can block alfa glukosidase that slow down the carbohydrate absorption in the abdomen. Kaemferol in *Z. zerumbet* rhizome also has effect on decreasing sugar level (Gaikwad *et al.*, 2014). Kaemferol can halt apotheosis and aspase-3 in sel β-pancreas with hiperglikemik condition. Furthermore, it can increase IL-1β, TNF-α, peroksidasi lipid and nitrat (Vinayagam dan Xu, 2015). Hence, the insulin sensitivity is increase. Cork fish contain albumin that improve beta cell pancreases (Aisyatussoffi dan Abdulgani, 2013). There is alfa cell I lagerhan island in which produce glucogen and beta cell producing

insulin. Albumin works as antioxidan and will be bound with radical hidrocil produced by Fe reaction with H_2O_2 (Roche *et al*., 2008).

The combination between Z. zerumbet extract and cork powder increase anti-diabetes effectiveness seen from %PKGD with same effectiveness as positive control group. However, the long term impact of both extracts use is unknown. Therefore, further research on its toxicity dose is needed.

CONCLUSIONS

Z. zerumbet rhizome ethanolic extract and *C. Striata* fish powder had antidiabetic activity at dose 300 mg/kgBB. The combination of the both ingredient based on statistical test did not differ significantly when compared with positive control in lowering blood sugar level.

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