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ICB Pharma 2015
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OP	E012

EFFECTIVENESS OF ETHANOLIC EXTRACT OF SARANG SEMUT (*Myrmecodia tuberosa* (non Jack) BI.) TO DECREASE BLOOD GLUCOSE LEVELS IN DIABETIC RATS INDUCED BY ALLOXAN

Tanti Azizah Sujono[1*], Sartika Nurhaini[1], E. M. Sutrisna[1], Sri Setiyani[1], Erwin Susanti[1]
[1]*Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia*

*Email : tanti_azizah@ums.ac.id

Abstract

Sarang semut (Myrmecodia tuberosa (non Jack) BI.) contain active compounds such as flavonoids, tannins, polyphenols, tocopherols, and minerals. Flavonoid in Sarang semut suspected can lower blood glucose levels by mechanism of stimulating insulin-secreting pancreatic β cells. The purpose of this study was to determine the effect of ethanolic extract of sarang semut to decrease blood glucose levels in rats induced by alloxan. This research used pretest and posttest with control design method. Before treatment, blood was taken from tail vein lateralis rats to measurement of glucose levels (baseline). Later, rats were induced by alloxan 160 mg/kgBW (intraperitoneal) to make diabetic in rats. Three days later after induced by alloxan, blood glucosa was measured. Twenty male of rats with glucose levels ≥ 200 mg/dL were used to research (as pretest) and divided into 5 groups. The first group (negative control) was treated with CMC Na 0,5%. Second group was treated with glibenclamide 0,5 mg/kgBW (positive control). The third to fifth groups were treated with ethanolic extract of sarang semut with dose 400 mg/kgBW, 800 mg/kgBW, and 1600 mg/kgBW. The blood were taken on days 0, 3, 5,10,14 and 21 (since pretest). The serum added to reagen kit glucose GOD FS from Diasys and readable the glucose levels used a spectrophotometer visible (λ 500 nm). Blood

glucose levels were tested by ANOVA followed by Post Hoc test (Tukey test). The result showed that ethanolic extract of Sarang semut with dose 400; 800 and 1600 mg/kgBW respectively could reduce significant ($p<0,05$) the blood glucose level and they had similar effect compared with positive control.

Keywords: *Myrmecodia tuberosa* (non Jack) Bl., Blood glucose, ethanolic extract, alloxan.

INTRODUCTION

Diabetes mellitus (DM) is a disease caused by the pancreas that produce insulin does not work in both absolute terms and relative to an increase in blood sugar levels (Subekti et al, 2005). According to WHO, people with diabetes (DM) in Indonesia in 2000 reached 8.4 million, and by 2030 is expected to be 21.3 million people (PERKENI, 2011). If blood glucose levels are not controlled in the long term, it can lead to the occurrence of diabetic complications.

Management of therapy in diabetes mellitus can be done in various ways such as regular exercise, a diet low in sugar, and also use oral hypoglycemic drugs or insulin (PERKENI, 2011). Today the use of drugs of herbal ingredients in medicine is increasing, but generally still little scientific evidence, while the demands of scientific evidence of users of traditional medicine is increasing with the increasing public awareness of health (Suharmiati, 2003).

Sarang semut grows in the highlands of Papua as epiphytic plants that used as traditional medicine. Sarang semut contains flavonoids, tannins, polyphenols, tocopherols also rich in various minerals (Subroto and Saputro, 2006). Study of Jelly & Makiyah (2011) showed that infusion Sarang semut (*Hydnophytum formicarum*) can increase the diameter and number of β cells of the islets of Langerhans in rats induced by alloxan 130 mg/ kgBW. Sarang semut dose 5.04 g/kgBW can increase the size of the diameter of the islets of Langerhans significantly from (33.46 ± 12.38) μm become (62.2 ± 15.88) μm. besides, H. formicarum 5.04 g/kgBW able to increase significantly the number of β cells of (14.4 ± 5.01) to (34.6 ± 3.81). So Sarang semut shown to reduce damage to the pancreas.

In addition, the ethanol extract of Sarang semut (*Myrmecodia pendens* Merr & Perry) concentration of 8.4%$^{w/v}$; 1 mL/30 gBW had hypoglycemic effect significant compared to the negative control with the rate of decline 17.06 mg/dL.hour in mice induced glucose 15%$^{w/v}$ (Taebe et al, 2012).

In the previous study showed that flavonoids in Sarang semut have activity as an antidiabetic. In this study use ethanol 70% which can sum up flavonoids. Besides, ethanol

70% non-toxic, selective in producing the optimal amount of active compound, can be mixed with water in any ratio and less heat is needed for concentration (Depkes RI, 1986).

Therefore it is necessary to study on the efficacy of ethanolic extracts of Sarang semut on the hypoglycemic effect in alloxan-induced diabetic rats. This research is expected to prove the efficacy of Sarang semut in lowering blood glucose levels.

MATERIALS AND METHODS

Instruments: scales of test animals (Triple Beam Balance), analytical balance, UV-Vis spectrophotometer (Stardust FC15), minispin (Eppendrof), vortex, micropipette, cuvette, blue tip, yellow tip, injection syringe (Terumo), oral needle.

Materials: Sarang semut (Papua), alloxan monohydrate (Sigma Aldrich), distilled water, CMC Na, reagent kit GOD FS (DiaSys).

Animal testing: healthy Wistar male rats, aged 2-3 months, weighing 150-250g.

The course of study:

Preparation of ethanolic extract of Sarang semut

One kg of crude Sarang semut was maserated in maserator, add 5 liters of ethanol 70%, then macerated for 2 days. Maserat was filtered and dregs back macerated for 2 days with 5 liters of ethanol. Maserat collected then concentrated using a rotary evaporator to obtain extract.

Preparation of CMC Na 0.5% solution

0.5g CMC Na put in 50 ml of hot distilled water and then added ad 100 ml with cold distilled water, stirred until homogeneous. CMC Na 0.5% solution was used to suspend the ethanolic extract of Sarang semut.

Preparation of alloxan

320 mg of alloxan monohydrate weighed, and then dissolved in 25 ml water for injection (WFI), stirred until homogeneous and immediately injected into rats.

Making of rat model of hyperglycemia

Rats were induced diabetic by Alloxan with dose 160 mg/kgBW, injected intraperitoneally (ip) (Chougale et al., 2007). On the third day the rats with blood glucose levels ≥ 200 mg / dL were used for research.

Determination of the dose of glibenclamide

Glibenclamide dose in rats is 0.5 mg/kgBW (Jelly and Makiyah, 2011). Dosage is based on the human (70 kg) therapeutic dose of glibenclamide is 5 mg. Then converted the human therapeutic dose of 70 kg to rats of 200 g = 0.018, so the dose for rats were 0.09 mg/200 gBW = 0.45 mg/kgBW ~ 0.5 mg/kgBW.

Test the effect of antidiabetic Sarang semut

Twenty rats were adapted in the laboratorium for 1 week. Rats were fasted for 6 hours with distilled water were given ad libitum, then measured their blood glucose levels. Then rats induced by alloxan with dose 160 mg/kgBW (ip). On the third day glucose levels were measured, and the rats that have glucose levels ≥ 200 mg/dL were used for research. The hyperglycemia rats were given treatment following groups:

I : CMC-Na 0.5% (negative control)

II : Glibenclamide 0.5 mg/kgBW (positive control)

III : Ethanolic extract of Sarang semut 0.4 g/kgBW

IV : Ethanolic extract of Sarang semut 0.8 g/kgBW

V : Ethanolic extract of Sarang semut 1.6 g/kgBW

This treatment is administered orally for 21 days. Before the blood drawn, rats were fasted 6 hours and blood samples were taken from the lateral tail vein subsequently measured blood glucose levels with GOD FS reagent (Dyasis) on days 0, 7, 14 and 21 to determine the hypoglycemic effect of ethanolic extracts of Sarang semut.

Data analysis

Data blood glucose levels on days 0, 7, 14, and 21 were tabulated and calculated percent decline in blood glucose levels (PKGD) with the formula:

$$\%PKGD = \frac{(\bar{x} \text{ blood glucose levelsnegative control}) - (\text{glucosa level post test})}{\bar{x} \text{ blood glucose level negative control}} \times 100\%$$

Distribution data of blood glucose and % PKGD were tested by Shapiro Wilk test and Levene's test for homogeneity. Because the data were normal then analyzed by one way ANOVA and followed by posthoc LSD test with a level confidence of 95%.

RESULTS AND DISCUSSION

The ethanolic extract of Sarang semut in this study were made by maceration method. The ethanolic extract of Sarang semut in this study were made by maceration method and the rendemen about 1.60%.

Test antidiabetic effects of ethanolic extracts of Sarang semut

Baseline glucose levels checked to ensure that the rats were used for the study had normal blood glucose levels of 50-125 mg /dL (Johnson and Delaney, 1996). After this the rats were injected alloxan 160 mg/kgBW (Chougale et al., 2007). Three days later the blood glucose levels were measured, if the glucose level ≥200 mg/dL, the rats used for research. Blood glucose levels were measured 4 times on days: 0, 7,14 and 21 (Table 1, Figure 1).

Table 1. Blood glucose level before and after treatment (n=4)

Treatment	Baseline (mg/dL)	Blood glucose levels (mg/dL) on days :			
		0	7	14	21
Negative control (aquadest)	125 90 113 105	211 242 244 236	252 245 212 216	248 260 248 256	291 270 281 272
($\bar{x}$ ± SD)	108,25±14,68	233,25±15,22	231,25±20,19	253,00±6,00	278,50±9,61
Positive control (glibenklamid 0,5 mg/kgBW)	109 94 96 114	208 230 236 224	197 187 193 103	132 107 101 122	110 93 89 80
($\bar{x}$ ± SD)	103,25±9,78	224,50±12,04	170,00±44,86	115,50±14,11	93,00±12,57
Ethanolic extract 0.4 g/kgBW	99 84 108 127	241 205 256 237	244 225 152 129	186 162 110 98	189 144 167 105
($\bar{x}$ ± SD)	104,50±17,97	234,75±21,45	187,50±55,62	139,00±41,87	151,25±35,89
Ethanolic extract 0.8 g/kgBW	96 117 132 128	243 222 248 250	241 133 260 252	127 102 167 193	114 128 142 119
($\bar{x}$ ± SD)	118,25±16,13	240,75±12,84	221,50±59,51	147,25±40,58	125,75±12,28
Ethanolic	85	224	58	86	79

extract 1.6	93	239	128	102	63
g/kgBW	114	218	112	116	87
	129	206	132	119	105
($\bar{x}$ ± SD)	105,25±20,01	221,75±13,72	107,50±34,11	105,75±15,11	83,50±17,46

Figure 1 shows that ethanolic extracts of Sarang semut able to lower blood glucose levels. While the negative control was not able to lower blood glucose levels proved on day 21 the rats still hyperglicemia (278.50 ± 9.61) mg/dL.

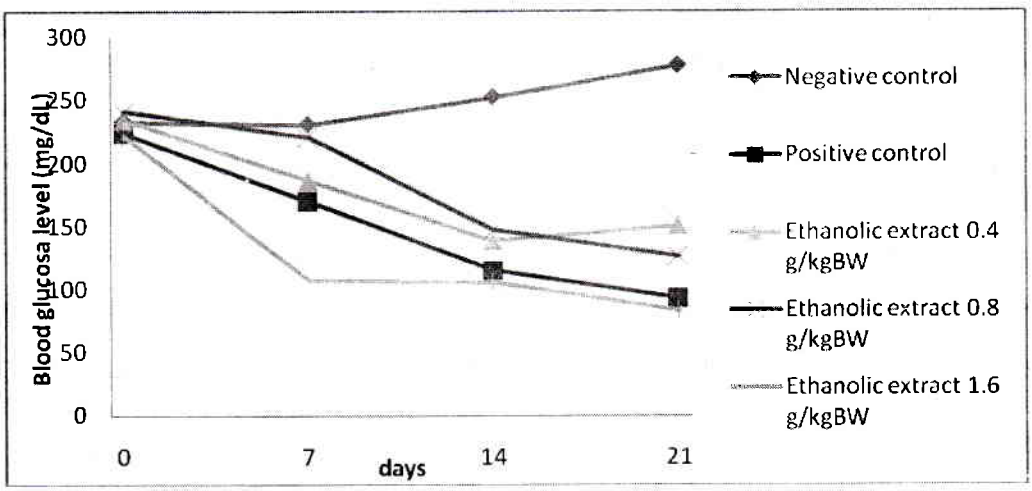

Figure 1. Blood glucose levels after treatment ethanolic extract of Sarang semut

Based on data from blood glucose levels (Table 1), then calculated %PKGD on day 21. Blood glucose levels on day 21 and % PKGD were tested normality with the Shapiro Wilk, because data normally distributed ($p>0.05$), then tested ANOVA and there were significant difference ($p<0.05$). The results of ANOVA test is used to see the differences among 5 groups of treatment in lowering blood glucose, they are the positive control, negative control and ethanolic extract dose of 0.4; 0.8 and 1.6 g/kgBW on day 21 after treatment

Table 2. Persentase PKGD after treatment ethanolic extract of Sarang semut on days 21

Treatment	% PKGD ($\bar{x}$ ± SD)
Negative control	0.37 ± 2,79
Positive control	66.61 ± 4.51
Ethanolic extract 0.4 g/kgBW	45.69 ± 12.89
Ethanolic extract 0.8 g/kgBW	54.85 ± 4.41
Ethanolic extract 1.6 g/kgBW	70.02 ± 6.27

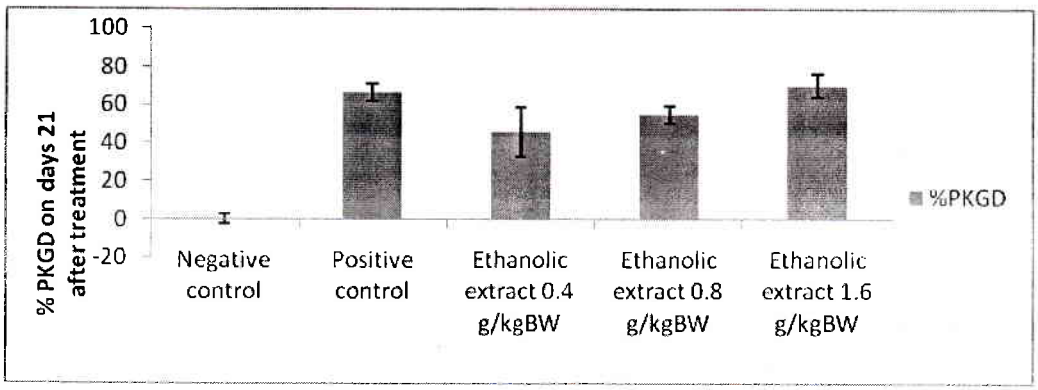

Figure 2. Persentase PKGD after treatment Ethanolic extract of Sarang semut

Results of LSD (Least Significant difference) test, levels of blood glucose (table 1) and % PKGD on day 21 (table 2) showed that the negative control was significantly different from the positive control and groups of ethanolic extract 0.4; 0.8 and 1.6 g/kgBW ($p<0.05$), this means glibenclamide and extracts of Sarang semut had hypoglycemic effects (antidiabetic). While LSD test results between the positive control (glibenclamide) and extract of Sarang semut dose of 1.6 g/kgBW did not differ significantly ($p>0.05$), it means the ethanolic extract of Sarang semut 1.6 g/kgBW able to lower blood glucose levels equivalent to glibenclamide. Whereas LSD test results between glibenclamide and extracts of Sarang semut 0.4 and 0.8 g/kgBW were significantly different, this means Sarang semut dose of 0.4 and 0.8 g/kgBW able to lower blood glucose levels but the effect is smaller than positive control (glibenclamide).

Flavonoids are thought to be responsible for the hypoglycemic effect. Prachayasittikul et al (2008) suggest flavonoid contained in the Sarang semut (*Hydnophytum formicarum*) namely isoliquiritigenin, butein, and protocatechualdehyde which can protect the integrity of pancreatic β cells. Based on previous research in Sarang semut (Myrmecodia pendens) contains a variety of flavonoids such as quercetin, luteolin, rutin, apigenin, kaempferol (Engida et al, 2012), as well as tannin, α-tocopherol, and stigmasterol were able to control blood glucose levels by stimulating insulin secretion of pancreatic β cells, inhibit glucose absorption in the intestine by the enzyme α-glucosidase inhibition, increases the solubility of blood glucose to be easily excreted through urine. Flavonoids as antioxidants are also able to reduce lipid metabolism disorders and kidney damage, inhibit oxidative damage to proteins and β cells, and increasing the sensitivity of insulin receptors (Taebe et al., 2012).

Based on research Sandhar et al (2012) flavonoid quercetin work on enzyme aldose reductase to reduce glucose into sorbitol that were oxidized to fructose, regenerate the pancreas gland, increases insulin secretion, increase Ca^{++} ion uptake of isolated islet

cells. According to Kim et al study (2000) flavonoid luteolin is able to inhibit α-glucosidase by 36%, so the absorption of glucose in the intestine is blocked, while according Tadera et al (2005) flavonoid apigenin and kaempferol able to inhibit the enzyme α-glucosidase.

CONCLUSION

Ethanolic extract of Sarang semut dose of 0.4; 0.8 and 1.6 g/kgBW had hypoglycemic effect in rats induced by alloxan.

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OP	E014

THE EFFECT OF LONG TERM ADMINISTRATION OF ETHANOLIC EXTRACT OF *Persea americana* Mill. PEEL TOWARDS ALKALINE PHOSPHATASE CONCENTRATION IN WISTAR MALE RATS INDUCED BY CARBON TETRACHLORIDE

Lusia Drikti Nini Gorantokan[1*], Phebe Hendra[1]

[1]*Faculty of Pharmacy Sanata Dharma University Yogyakarta, Yogyakarta, Indonesia*

*Email: lusia.drikti15@gmail.com

Abstract

Persea americana Mill. seed and peel contain flavonoid that acts as an antioxidant. *Persea americana* Mill. seed has the ability to decrease alanine transaminase (ALT) enzyme in Wistar male rats induced by carbon tetrachloride as a hepatotoxin. This present research was conducted to get information about the effect of long term administration of *Persea americana* Mill (*P. americana*) peel toward alkaline phosphatase (ALP) concentration in male Wistar rats induced by carbon tetrachloride, and get information about relation of ethanolic extract of *P. americana* peel doses. This research used thirty Wistar male rats weight arround 150-250 g and divided to six treatment groups. Group I (hepatotoxin control) was given carbon tetrachloride 2 ml/kgBW. Group II (negative control) was given 100% *olive oil* 2 ml/kg BW. Group III (ethanolic extract of *P. americana* peel) was given ethanolic extract of *P. americana* peel 1400 mg/kg BW. Group IV, V, and VI (treatment group) were given ethanolic extract of *P. americana* peel doses 0,35; 0,7; 1,4 g/kg BW orally, once daily for 6 days. On the seventh day, carbon tetrachloride 2 ml/kg BW was given to all treatment group, then 24 hours after induced by carbon tetrachloride, the blood of Wistar male rats were collected and measured the ALP concentration. The ALP concentration analysed statistically. From the result, can be concluded that long term administration of ethanolic extract of *P. americana* pell dose I and dose II (0,35 g/kg BW; 0,7 g/kg BW) didn't give the effect to alteration of ALP's concentration in Wistar male rats induced by carbon tetrachloride, whereas dose III (1,4 g/kg BW) gave the alteration of ALP's concentration in Wistar male rats induced by carbon tetrachloride. There were no doses relation with ALP concentration.

Keywords: *Persea americana* Mill., peel, ethanolic extract, carbon tetrachloride, alkaline phosphatase.

INTRODUCTION

Liver is the largest organ in the body. Liver has the trilateral shape and rough, located underneath diaphragma, over the abdominal cavity. Liver has the function as toxic detoxification, to excretion the unimportant compound from the body, as the cobalamine