

## Anti-diabetic effect of a combination of andrographolide-enriched extract of *Andrographis paniculata* (Burm f.) Nees and asiaticoside-enriched extract of *Centella asiatica* L. in high fructose-fat fed rats

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Traditionally, a combination of medicinal plants is commonly used for lowering blood glucose in diabetic patients in order to provide additional benefits of the single drug. *A. paniculata* and *C. asiatica* are two traditional medicines from South Asian and Southeast Asian countries consumed by people for treating diabetes mellitus and its complications. Hyperglycemia in the rats was stimulated by high fructose-fat diet that consists of 36% fructose, 15% lard, and 5% egg yolks in 0.36 g/200 g body weight for 70 days. The rats were orally administered with the combination of andrographolide-enriched extract of *A. paniculata* (AEEAP) leaves and asiaticoside-enriched extract of *C. asiatica* (AEECA) herbs from day 70 for 7 days. Antidiabetic activity was evaluated by estimating mainly the blood glucose levels and other parameters such as HDL, LDL, cholesterol and triglyceride. The results showed that combination at the ratio of 70:30 exhibited a promising antidiabetic effect in high-fat-fructose-fed rat, and exhibited synergistic effects on blood cholesterol and HDL levels. It can be concluded that its antidiabetic effect was better than that of single treatment of AEEAP or AEECA. That combination was also potential to develop as a blood glucose-lowering agent for diabetic patients.

**Keywords:** *Andrographis paniculata*, *Centella asiatica*, Diabetes mellitus, Hyperlipidemia, High-fructose-fat diet

Diabetes mellitus (DM) is a metabolic disorder associated with hyperglycemia, abnormalities in carbohydrate, fat and protein metabolism. Based on dependence on exogenous insulin, DM is classified into two types: type 1 DM and type 2 DM<sup>1-3</sup>. Type 1 DM, also named insulin-dependent diabetes mellitus (IDDM), is related to an absolute deficiency of insulin and stimulated by a destruction of pancreatic cells due to autoimmune processes. Type 2 DM, non-insulin-dependent DM (NIDDM), is related to insulin resistance with compensatory. Uncontrolled type 2 DM can evolve into the type 1 DM so that the patients should consider the diet, lifestyle and oral hypoglycaemic drugs<sup>3-5</sup>.

Increasing number of type 2 DM patients in developing countries is very high. Modern lifestyle with high-fat diet increases risk factor for type 2 DM<sup>6</sup>. Nutrient consumption patterns have been shifted from a healthy traditional food (high-fiber, low-fat, low-calorie diet) toward increasing consumption of high-

calorie foods containing low fiber, high fats, red meats, and refined carbohydrates<sup>7</sup>. The uncontrolled condition can develop to be insulin resistance, a clinical condition of decrease in insulin potency to uptake glucose into cells. Insulin resistance is the early and main character of type 2 DM<sup>8</sup>. One approach for treating type 2 DM is the use of traditional medicine. To date, most medicinal plants in the form of single use has not shown satisfactory results. Therefore, the attempts to combine several antidiabetic drugs are being developed.

*Andrographis paniculata* (Burm. f.) Nees is a original Indian plant growing widely to Southeast Asian countries including Indonesia, Malaysia and Thailand. The ethanolic extract of *A. paniculata* was reported to decrease the blood glucose levels in both streptozotocin (STZ)-diabetic rats and high fructose-fat fed rats<sup>9,10</sup>. The plant has insulin-releasing actions on BRIN-BD11 cells, a pancreatic cell line expressing insulin and glucokinase<sup>11</sup>. The main active compound of the plant is andrographolide<sup>12,13</sup>. The compound also exhibited hypoglycaemic effects in STZ-diabetics rats obviously<sup>14</sup>. The compound increased the cellular uptake of glucose by increasing

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mRNA and protein levels of GLUT 4 in type 1 DM rats<sup>15</sup>. Andrographolide decreased the levels of blood glucose by increasing GLUT-4 expression in high-fat-fructose-fed rats<sup>16</sup>. In addition, the compound also succeeded to decrease the triglyceride and LDL levels in those rats<sup>10</sup>.

*Centella asiatica* L. is a wild plant growing widely in the area of India, China and Southeast Asian countries. In the Ayurvedic system of Indian traditional medicine, the plant is used for various pathological disorders especially for healing wound<sup>17</sup>. The plant was also used for wound healing promotion in diabetic wound patients<sup>18</sup>. In addition, the plant exhibited anti-diabetic activities in 3T3-L1 adipocytes and db/db mice<sup>19</sup>, a model of diabetic dyslipidemia<sup>20</sup>. It indicates that plant is promising to treat type 2 DM related to insulin resistance. The active compound of this plant is asiaticoside, a triterpene compound. The compound is reported to be responsible for its antioxidant-related healing wounds<sup>21</sup>. In addition, that compound also succeeded to up-regulate PPAR<sup>22</sup>, a potent insulin sensitizer receptor for the treatment of type 2 DM<sup>23</sup>.

Both *A. paniculata* and *C. asiatica* were scientifically reported as anti-diabetic herbs. Their active compounds suggested for their biological activities are andrographolide and asiaticoside, respectively. The mechanism of action of anti-diabetic activities are also different. In the present study, andrographolide-enriched extract of *A. paniculata* and asiaticoside-enriched extract of *C. asiatica* L. are combined and evaluated for their anti-diabetic effect in high fructose-fat fed rats, a type 2 DM rat model.

## Materials and Methods

**Materials**—Andrographolide, asiaticoside and metformin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carboxymethyl cellulose, fructose, glucose were obtained from E. Merck, Darmstadt, Germany. Glucose, cholesterol, triglyceride, LDL and HDL levels were measured using colorimetric methods (DiaSys, Diagnostic Systems GmbH, Holzheim, Germany).

**Animals**—Wistar rats (2-3 month old) weighing 150-200 g used in the study were maintained on a constant temperature ( $22 \pm 2$  °C) a constant relative humidity ( $55 \pm 10\%$ ) and automatically controlled 12:12 h light-dark cycle (light on at 07:00 hrs). They were fed with a standard laboratory food and water *ad libitum*. The animal handling protocols was

based on the guidelines of the animal care of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

**Preparation of andrographolide-enriched extract of *A. paniculata* leaves**—The leaves of *A. paniculata* were collected during June from the area around Sleman Yogyakarta, Indonesia. The plant was authenticated by a botanist at Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The voucher specimen was stored in a herbarium of the department. The dried leaves were powdered and stored in an airtight container for further use.

The leaves were dried and then ground to a powder. The powder of *A. paniculata* was macerated with 95% (v/v) ethanol overnight. After twice re-extraction, collected extract was evaporated under reduced pressure to provide of an viscous extract. The extract was fractionated gradually using *n*-hexane (ratio of 1:20) and ethyl acetate (ratio of 1:10). The insoluble fraction of *n*-hexane was fractionated with ethyl acetate. The insoluble fraction of ethyl acetate was obtained, and concentrated by rotary vacuum evaporator to obtain an viscous extract. Subsequently, that fraction was washed with hot water, and diluted with 95% ethanol to provide an andrographolide-enriched extract of *A. paniculata* (AEEAP) leaves. Final product was evaporated again to provide an viscous extract of AEEAP.

**Preparation of asiaticoside-enriched extract of *C. asiatica* herbs**—The herbs were collected during June from the area around Tawangmangu, Central Java Indonesia, and authenticated by a botanist at Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The herbs were shade dried, then powdered, and stored in an airtight container for the study. Preparation of asiaticoside-enriched extract of *C. asiatica* L. leaves was similar to preparation of AEEAP, however in this case ethyl acetate (ratio of 1:10) was used to extract the active compound asiaticoside. After collection, the obtained soluble fraction of ethyl acetate was concentrated by rotary vacuum evaporator to yield an asiaticoside-enriched extract of *C. asiatica* (AEECA) herbs.

**Experimental design**—The rats were conditioned to the experimental laboratory for 8 days for acclimatization. Hyperglycemia in the rat was induced by high fructose-fat administration. That diet was

prepared by mixing fructose (36%), lard (15%) and egg yolks (5%) in 0.36 g/200 g body weight ratio<sup>10</sup>. Control rats (normal rats) were fed with standard laboratory chow diet, and its composition was according to the preliminary study. That dietary diet was administrated for the total period of 77 days. The rats were treated with the extracts, their combination or test compound at day 70. During this period, rat body weight was monitored regularly. The high fructose-fat fed rats were randomly assigned into following 7 groups of 6 each :

- Gr. 1 : rats received oral saline 10 mL/kg body weight (control group).  
 Gr. 2 : rats received andrographolide-enriched extract of *A. paniculata* leaves (AEEAP) (1303.8 mg/kg body weight, po, once daily).  
 Gr. 3 : rats received asiaticoside-enriched extract of *C. asiatica* herbs (AEECA) (1000 mg/kg body weight, po, once daily).  
 Gr. 4 : rats received a combination of AEEAP (912.1 mg/kg body weight) and AEECA (300 mg/kg body weight) with a ratio of 70:30 (w/w), orally.  
 Gr. 5: rats received a combination of AEEAP (651.5 mg/kg body weight) and AEECA (500 mg/kg body weight) with a ratio of 50:50 (w/w), orally.  
 Gr. 6 : rats received a combination of AEEAP (390.9 mg/kg body weight) and AEECA (700 mg/kg body weight) with a ratio of 30:70 (w/w), orally.  
 Gr. 7 : rats received metformin dose 45 mg/kg body weight orally, once daily.

The rats received AEEAP, AEECA, their combination with various doses or metformin (positive control) at the day 70 onwards for the next 7 days. The blood samples were collected from 8-10 h fasted rats by means of capillary tubes through retro-orbital plexus at the day of 0 (basal value), 20, 30, 50 and 70. The blood samples were centrifuged at 3000 g for 10 min. The serum was collected for

determination of glucose, LDL-cholesterol, HDL-cholesterol, total cholesterol, and triglyceride levels using colorimetric methods.

*Statistical analysis*—The data were analysed as mean±SE. Statistical analysis of the data used one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. *P* values of less than 0.05 were considered as significant.

## Result

*Effect of high-fructose-fat diet*—To induce the diabetic condition, the rats were treated with high-fructose-fat diet containing a mixture of 36% fructose, 15% lard and 5% egg yolks in 0.36 g/200 g body weight for 70 days. The diet could increase the glucose level by 66% in comparison to that of the normal rats value (Table 1). The lipid parameters were also elevated after 70-day high-fructose-fat-diet administration. The diet significantly increased the cholesterol, trygliseride and LDL levels by 27, 101 and 46%, respectively. In addition, the diet suppressed the HDL level by 41% significantly.

The effect of glibenclamide, an sulfonylurea antidiabetic drug, was also evaluated in high fructose-fat fed rats. In the normal rats, glibenclamide obviously decreased the blood glucose levels with hypoglycemic activity of 65.39±2.20% (Fig. 1). However, the hypoglycemic activity of glibenclamide in high fuctose-fat fed rats was 31.87±0.57%. It indicates that the hypoglycaemic activity of glibenclamide decreased when administered to the high fuctose-fat fed rats. Metformin, an extrapancreatic antidiabetic drug, obviously decreased the blood glucose levels in high fuctose-fat fed rats by 65.30±3.61%.

*Effect of the extracts and their combinations on blood glucose levels*—Both extracts, AEEAP (1.303 mg/kg body weight) and AEECA (1 g/kg body weight), markedly decreased the blood glucose

Table 1—Comparison of glucose, cholesterol, trygliseride, LDL and HDL levels on the day 0 and the day 70 between normal rats and high fructose-fat fed rats

[Values are mean±SE from 10 rats each]

Parameter (mg/dL)	Normal rats		High fructose-fat fed rats	
	Day 0	Day 70	Day 0	Day 70
Glucose	74.90±6.10	88.89±5.52	73.45±7.72	147.70±6.67*
Cholesterol	65.94±2.51	68.67±2.47	65.56±3.22	87.00±2.54*
Triglycerides	96.02±3.30	137.17±13.43	99.85±15.37	276.00±43.41*
LDL	47.80±4.46	51.00±4.34	41.09±4.00	74.33±3.42*
HDL	119.32±5.61	111.50±6.03	115.37±3.77	68.50±3.50*

\**P* < 0.05

levels in high fuctose-fat fed rats (Table 2). The hypoglycaemic effect of both extracts were 61.21 and 60.12%, respectively. Their combination also lowered the blood glucose levels in high fuctose-fat fed rats significantly. At combination ratio of 70:30; 50:50; 30:70 (w/w) decreased blood glucose by 68.13, 46.16 and 40.36%, respectively. Metformin, an biguanid antidiabetic drug, decreased the blood glucose level by 62.63%.

*Effect of the extracts and their combinations on cholesterol and triglyceride levels*—All the combinations (1-3) were able to lower the blood cholesterol in high fuctose-fat fed rats by 85.66; 67.37 and 65.60%, respectively (Table 3). AEEAP dose 1.303 mg/kg body weight and AEECA dose

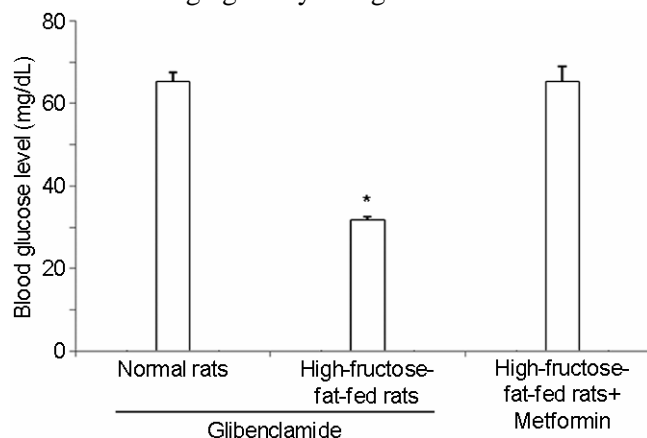


Fig. 1—Hypoglycemic activity of glibenclamide in normal rats and in high-fructose-fat rats. In addition, metformin was used as positive control in hypoglycemic activity test in insulin resistant rats. Each value is a mean±SE from 3 observations. \* $P < 0.05$ , compared to the normal rats (rats fed normal chow).

1 g/kg body weight obviously decreased the blood cholesterol by 68.29 and 64.14%, respectively. The effect of metformin was less potent than that of the extracts.

All treatments (Groups 2-7) exhibited mild lowering effects on the triglyceride levels in high fuctose-fat fed rats (Table 4). The combination 1 exhibited potent triglyceride-decreasing effect, however still less potent than that of metformin.

*Effect of the extracts and their combinations on LDL and HDL levels*—All the combinations (1-3) and all extracts exhibited mild lowering effects on the triglyceride levels in high fuctose-fat fed rats (Table 5). Those triglyceride-lowering effects range 10-20%. Metformin showed most potent effect on the LDL levels by 38.58%.

AEEAP (1.303 mg/kg body weight) and AEECA (1 g/kg body weight), increased the blood triglyceride levels in high fuctose-fat fed rats by 39.92 and 31.03% (Table 6). Combination 1-3 exhibited the HDL-increasing effect by 71.71, 45.22 and 44.91%. Metformin exhibited most potent effect on the HDL levels by 80.21%.

## Discussion

*A. paniculata* has exhibited potent antidiabetic activities in various diabetic animal models<sup>10,14,16</sup>. Its activities were contributed by its active compounds mainly andrographolide. In addition, andrographolide is a major constituent and abundance in the plant<sup>12,13</sup>. The compound reported to exhibit hypoglycemic effects in both type 1 and 2 DM rats models<sup>10,14</sup>. It indicates that the compound contributes for the

Table 2—Effect of AEEAP, AEECA, their combination with various doses or metformin (positive control) on the blood glucose level in high fructose-fat fed rats rats

[Values are mean±SE from 6 rats each]

Group	Treatment	Blood glucose (mg/dL)			Percentage of decrease (%)
		Day-0	Day-70	Day-77	
I	Control (saline)	77.54 ± 2.48	129.58 ± 1.43	131.02 ± 1.96	-3.24 ± 2.78
II	AEEAP	71.71 ± 4.64	133.03 ± 4.02	97.53 ± 2.72	61.21 ± 6.02*
III	AEECA	73.38 ± 5.75	134.70 ± 7.83	98.29 ± 3.94	60.12 ± 5.19*
IV	Combination 1	74.67 ± 3.79	133.72 ± 4.89	96.47 ± 5.13	68.13 ± 9.24*
V	Combination 2	74.41 ± 8.26	137.57 ± 4.01	109.28 ± 3.72	46.16 ± 4.62*
VI	Combination 3	73.73 ± 5.43	131.28 ± 7.59	108.24 ± 3.39	40.36 ± 3.70*
VII	Metformin	70.35 ± 2.39	135.19 ± 3.43	95.84 ± 4.78	62.63 ± 7.90*

\*  $P < 0.05$

AEEAP : andrographolide-enriched extract of *A. paniculata* leaves at dose 1.303 mg/kg body wt.

AEECA : asiaticoside-enriched extract of *C. asiatica* herbs at dose 1 g/kg body wt.

Combination 1, 2, 3 : combinations of AEEAP and AEECA with a ratio of 70:30; 50:50; 30:70 (w/w), respectively.

Table 3—Effect of AEEAP, AEECA, their combination with various doses or metformin (positive control) on the blood cholesterol level in high fructose-fat fed rats

[Values are mean±SE from 6 rats each]

Group	Treatment	Blood cholesterol (mg/dL)			Percentage of decrease (%)
		Day-0	Day-70	Day-77	
I	Control (saline)	67.43 ± 2.48	81.17 ± 5.13	82.83 ± 4.75	-24.28 ± 8.76
II	AEEAP	60.23 ± 1.72	79.67 ± 2.22	66.83 ± 2.55	68.29 ± 6.89*
III	AEECA	66.74 ± 2.39	77.83 ± 1.23	70.17 ± 2.30	64.14 ± 6.74*
IV	Combination 1	65.56 ± 3.22	85.33 ± 2.08	68.17 ± 3.08	85.66 ± 3.06*
V	Combination 2	61.52 ± 1.56	83.17 ± 2.99	69.17 ± 2.02	67.37 ± 6.07*
VI	Combination 3	60.51 ± 1.91	79.33 ± 1.48	68.00 ± 1.51	65.60 ± 8.40*
VII	Metformin	67.11 ± 2.80	82.67 ± 3.40	78.00 ± 2.82	29.23 ± 2.32*

\*  $P < 0.05$

Table 4—Effect of AEEAP, AEECA, their combination with various doses or metformin (positive control) on the blood triglyceride level in high fructose-fat fed rats

[Values are mean±SE from 6 rats each]

Group	Treatment	Blood triglycerides (mg/dL)			Percentage of decrease (%)
		Day-0	Day-70	Day-77	
I	Control (saline)	82.66 ± 5.95	269.95 ± 9.80	281.33 ± 9.61 <sup>#</sup>	-6.61 ± 2.53
II	AEEAP	84.91 ± 3.41	262.17 ± 2.07	245.33 ± 3.86	9.49 ± 1.31*
III	AEECA	87.72 ± 0.92	283.83 ± 8.62	266.17 ± 7.76	8.97 ± 0.77*
IV	Combination 1	84.14 ± 0.83	289.33 ± 7.61	266.33 ± 7.00	11.23 ± 0.84*
V	Combination 2	84.00 ± 0.87	261.83 ± 5.26	243.33 ± 2.77	10.12 ± 2.00*
VI	Combination 3	88.41 ± 1.95	288.83 ± 8.86	272.00 ± 6.20	8.08 ± 1.45*
VII	Metformin	82.21 ± 3.24	287.50 ± 6.67	254.33 ± 6.59	16.27 ± 0.76*

\*  $P < 0.05$

Table 5—Effect of AEEAP, AEECA, their combination with various doses or metformin (positive control) on the blood LDL level in high fructose-fat fed rats

[Values are mean±SE from 6 rats each]

Group	Treatment	Blood LDL (mg/dL)			Percentage of decrease (%)
		Day-0	Day-70	Day-77	
I	Control (saline)	40.83 ± 1.82	67.38 ± 5.42	68.67 ± 5.09	-11.09 ± 7.17
II	AEEAP	40.17 ± 2.05	65.24 ± 2.71	60.50 ± 3.12	18.92 ± 5.46*
III	AEECA	40.50 ± 1.66	64.02 ± 3.32	59.33 ± 3.17	19.91 ± 4.09*
IV	Combination 1	43.83 ± 1.66	65.67 ± 5.74	61.67 ± 3.02	13.95 ± 2.92*
V	Combination 2	45.75 ± 1.30	74.67 ± 2.92	71.00 ± 2.91	12.84 ± 2.53*
VI	Combination 3	41.09 ± 4.00	74.33 ± 3.42	70.33 ± 2.99	12.59 ± 1.31*
VII	Metformin	41.83 ± 4.29	63.40 ± 6.74	52.17 ± 2.93	38.58 ± 7.31*

\*  $P < 0.05$

antidiabetic activities of *A. paniculata*. In the present study, the ethanolic extract of *A. paniculata* was then fractionated gradually using *n*-hexane and ethyl acetate, respectively to provide insoluble fraction of ethyl acetate. The insoluble product was diluted with ethanol 90% to yield an andrographolide-enriched extract of *A. paniculata* (AEEAP) leaves.

*C. asiatica* grows widely in many areas in South and Southeast Asian countries. The active constituents of *C. asiatica* are triterpenoids, which are asiaticosides (mainly), asiatic acid, madecassoside and madasiatic acid<sup>24</sup>. In the *in vitro* and *in vivo* studies, the plant showed promising anti-diabetic activities<sup>19</sup>. Based on those facts, that plant is

Table 6—Effect of AEEAP, AEECA, their combination with various doses or metformin (positif control) on the blood HDL level in high fructose-fat fed rats

[Values are mean±SE from 6 rats each].

Group	Treatment	Blood HDL (mg/dL)			Percentage of increase (%)
		Day-0	Day-70	Day-77	
I.	Control (Saline)	117.29 ± 1.30	83.33 ± 5.16	83.17 ± 4.54	-2.86 ± 6.03
II.	AEEAP	117.15 ± 1.79	73.17 ± 4.04	90.50 ± 5.19	39.92 ± 8.20*
III.	AEECA	116.32 ± 6.25	72.67 ± 8.01	86.67 ± 5.56	31.03 ± 3.18*
IV.	Combination 1	108.24 ± 1.52	74.00 ± 2.94	99.00 ± 3.02	71.72 ± 7.36*
V.	Combination 2	109.90 ± 3.99	75.50 ± 5.01	89.83 ± 4.07	45.22 ± 4.97*
VI.	Combination 3	114.03 ± 8.25	73.00 ± 2.66	89.50 ± 2.13	44.91 ± 7.51*
VII.	Metformin	115.37 ± 3.77	71.83 ± 1.94	106.83 ± 4.19	80.21 ± 3.61*

\*  $P < 0.05$ 

promisingly used for treating type 2 DM related to insulin resistance. In the present study, its ethanolic extract was also fractionated gradually using *n*-hexane and ethyl acetate. Soluble fraction of ethyl acetate was condensed to obtain an asiaticoside-enriched extract of *C. asiatica* (AEECA) herbs.

In the present study, the antidiabetic activity of the combination of AEEAP and AEECA was evaluated in high fructose-fat fed rats, a type 2 DM rat model. Long-term high-fructose administrations in animals was often used for inducing type 2 DM related to an insulin resistance<sup>26,27</sup>. The high fructose-fat fed can stimulate insulin resistance, hyperinsulinemia, and dyslipidemia<sup>10,16,28</sup>. Long-term period increase in insulin (hyperinsulinemia) can decrease the insulin sensitivity<sup>26,27</sup>. Hyperinsulinemia can influence the characteristic of adipocyte secretion that is responsible for insulin resistance in both adipocytes and myocytes<sup>28</sup>. In addition, high accumulation of fructose in liver can evoke the lipogenesis and triglyceride accumulation rapidly. Subsequently, those conditions can reduce the insulin sensitivity, and increase the VLDL formation<sup>29</sup>. In present the study, the administration of high-fructose-fat-diet with composition of 36% fructose, 15% lard, and 5% egg yolks for 70 days could increase the blood glucose, cholesterol, triglyceride and low-density lipoprotein (LDL) levels by 66, 27, 100 and 46%, respectively. In addition, that treatment suppressed the blood high-density lipoprotein (HDL) levels in rats. HDL is a smallest lipoprotein that bind lipids (cholesterol and triglycerides) so that those lipid can be transported with the water-based bloodstream<sup>30</sup>.

Glibenclamide is an oral an hypoglycemic agent acting on the beta cells of pancreas directly (pancreatic effect). The drug stimulates the cell to

release insulin. However, the drug will be less effective in type 2 DM related to insulin resistance. In the present study, glibenclamide showed a three fold more potent hypoglycemic activity in normal rats than that in high fructose-fat fed rats. However, metformin succeeded to decrease the blood glucose levels in high fructose-fat fed rats. Metformin is an oral hypoglycaemic agent that acts to stimulate insulin-induced glucose uptake components such as GLUT-4 into skeletal muscle and adipocytes (extrapancreatic action)<sup>31</sup>.

In the present study, both AEEAP and AEECA could decrease the blood glucose, cholesterol, triglyceride and LDL levels, and increase the HDL level significantly (Table 2). Their combination especially at the ratio of 70:30 (AEEAP:AEECA) also succeeded to lower the levels of those first four parameters, and increase the HDL levels in high fructose-fat fed rats. The effects of that combination on blood glucose and triglyceride levels were only slightly higher than those of single treatment of AEEAP and AEECA. However, that combination exhibited more potent effect on blood cholesterol and HDL levels than those of the single treatment, even though that effect on LDL level was mildly.

Both *A. paniculata* and *C. Asiatica* exhibited antidiabetic effects in several studies<sup>9,10,19</sup>. *A. paniculata* contains an active compound, andrographolide, that exhibits hypoglycaemic effects in several study<sup>10,14</sup>. The compound potently lowered the blood glucose levels in streptozotocin-induced diabetics rats, a type 1 DM rat model<sup>14</sup>. Andrographolide stimulated the glucose uptake into the isolated-soleus muscle of streptozotocin-diabetics rats by increasing muscle GLUT4 protein and mRNA<sup>32</sup>. The compound also suppressed the level of blood glucose and lipids levels

in high fat-fructose fed rat<sup>10</sup>. On the other hand, *C. Asiatica* exhibited anti-diabetic activities in 3T3-L1 adipocytes and db/db mice, a model of diabetic dyslipidemia<sup>20</sup>. Reportedly, its active compound asiaticoside up-regulated PPAR<sup>22</sup>, a potent insulin sensitizer receptor for the treatment of type 2 DM related to insuline resistance<sup>23,25</sup>.

### Conclusion

Based on the results, it can be concluded that the combination of andrographolide-enriched extract of *A. paniculata* leaves and asiaticoside-enriched extract of *C. asiatica* herbs at the ratio of 70:30 exhibited a promising antidiabetic effect in high-fat-fructose-fed rat. That combination also showed synergistic cholesterol-lowering and HDL-increasing activities.

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