

## The effects of combined medicinal plants infusion on blood glucose, cholesterol, and triglyceride levels in hyperglycemic *Sprague-Dawley* rats

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Received: September 5, 2015; Revised: September 30, 2015; Accepted: October 8, 2015

### Abstrak

**Latar belakang:** Kadar glukosa, kolesterol dan trigliserid darah yang tinggi dapat memicu terjadinya berbagai penyakit sehingga diperlukan cara untuk mengontrol ketiga parameter tersebut, salah satunya dengan obat tradisional. Penelitian dilakukan untuk membuktikan efek penurunan kadar glukosa, kolesterol dan trigliserid darah tikus *Sprague-Dawley* (SD) dari infusa kombinasi sambiloto, salam, kayu manis, dan temulawak.

**Metode:** Penelitian terhadap 3 kelompok uji (kontrol, perlakuan, dan metformin sebagai kontrol positif) dilakukan pada bulan Juni-Agustus 2014 di laboratorium hewan coba B2P2TOOT Jawa Tengah Central Java. Hewan uji adalah tikus galur SD usia?. Induksi hiperglikemik tikus uji dengan pemberian high fructose diet (HFD), yaitu campuran fruktosa (36%), kuning telur (20%), dan pellet standar (44%) dalam 0,36 g /200 g Berat Badan (BB) selama 70 hari. Setelah diperoleh tikus hiperglikemik pada hari ke-70, dilanjutkan pemberian infusa formula selama 7 hari. Ketiga parameter uji meliputi kadar glukosa, trigliserida, dan kolesterol total tikus uji hari ke-77 dibandingkan dengan hari ke-70. Pemeriksaan histopatologi organ pankreas dilakukan pada di akhir percobaan.

**Hasil:** Pemberian HFD selama 70 hari menyebabkan peningkatan signifikan kadar glukosa ( $p=0,0001$ ), kolesterol ( $p=0,001$ ) dan trigliserid total ( $p=0,006$ ) darah. Peningkatan berat badan tikus uji dibandingkan tikus kelompok kontrol tidak berbeda bermakna ( $p=0,792$ ). Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB menurunkan kadar glukosa, kolesterol, dan trigliserida darah tikus uji berurutan sebesar 37,09% 19,51%, dan 79,29%.

**Kesimpulan:** Pemberian infusa ramuan sambiloto, salam, kayu manis, dan temulawak dosis 491,4 mg/200 g BB selama 7 hari secara signifikan menurunkan kadar glukosa darah, kolesterol, dan trigliserida tikus hiperglikemik. (*Health Science Journal of Indonesia* 2015;6:99-104)

**Kata kunci:** hiperglikemik, tikus, glukosa, sambiloto

### Abstract

**Background:** High levels of blood glucose, cholesterol, and triglyceride tend to increase the incidence of several diseases. This study aimed to prove the effects of combined infusion of *sambiloto*, *salam*, *kayu manis*, and *temulawak* on decreasing blood levels of glucose, cholesterol, and triglyceride in *Sprague-Dawley* (SD) rats.

**Methods:** The rats were divided into 3 groups (control, treatment, and metformin as positive control). The study was conducted at the animal laboratory of MPTMRDC, Central Java, on June to September 2014. Hyperglycemia was induced by administering high fructose diet (HFD), a mixture of fructose (36%), egg yolk (20%), and standard pellets (44%) in 0.36 g/200 g Body Weight (BW) for 70 days. The combined infusion was given orally to the hyperglycemic rats for 7 consecutive days. The parameters were blood levels of glucose, cholesterol, and triglycerides. The pancreas was examined histopathologically at the end of the study.

**Results:** HFD for 70 days led to significant increase in glucose ( $p=0.0001$ ), cholesterol ( $p=0.001$ ), and total triglycerides ( $p=0.006$ ) levels, The increase of body weight of experiment group had no significant difference with control group ( $p=0,792$ ). as well as body weights. The combined infusion of 491.4 mg/200 g BW significantly reduced blood glucose, cholesterol, and triglyceride levels in rats by 37.09, 19.51, and 79.29 %, respectively.

**Conclusion:** The administration of the combined infusion with a dose of 491.4 mg/200 g BW for 7 consecutive days decreased blood glucose, cholesterol, and triglyceride levels in hyperglycemic rats. (*Health Science Journal of Indonesia* 2015;6:99-104)

**Keywords:** hyperglycemia, rat, glucose, *sambiloto*

Diabetes mellitus (DM) is a metabolic disorder characterized by high level of blood glucose, the presence of glucose in urine, along with symptoms of polyphagia, polydipsia, polyuria, blurred vision, weight loss, itching, tiredness, and sleepiness.<sup>1</sup> WHO data showed DM as the 6<sup>th</sup> leading cause of death in the world. Approximately 1.3 million people die from DM, with 4 % dying before the age of 70 years. The prevalence of DM in productive aged urban Indonesians is 4.6%, consisting of 1.1% diagnosed DM and 3.5% undiagnosed DM.<sup>2</sup> DM can be due to various factors, including insulin resistance, a condition in which the body's muscle, fat, and liver cells do not use insulin effectively, and other environmental factors.<sup>3</sup> If not handled properly, DM will cause complications both acute or chronic.<sup>4</sup> The long term use of medicine can cause negative effects especially on kidney function.

There are many medicinal plants which has been proven empirically as having antihyperglycemic properties, such as *salam* (*Syzygium polyanthum*) and *sambiloto* (*Andrographis paniculata* Ness). As reported by Nurwati (2009), *S. polyanthum* could lower blood glucose levels indicated by a significant difference between control and treatment group (dose of 4 g/100 g; p=0.000), characterized by a decrease of malondialdehyde (MDA) levels of alloxan-induced diabetes in male white rats.<sup>5</sup> Ethanolic extract of *S. polyanthum* leaves at concentrations of 30 and 70% exhibited hypoglycemic effects in glucose loaded rabbits.<sup>6</sup> With 35% leaves infusion, a decrease in glucose levels was reported in glucose loaded rabbits when compared to standard glibenclamide.<sup>7</sup>

Ethanolic extract of *Cinnamomum zeylanicum* cortex with a dose of 100 mg/kg BW/day for 90 consecutive days has been reported to increase the weight of reproductive organs, with increased motility and sperm count in Wistar rats.<sup>8</sup> *C. zeylanicum* with doses of 100, 200 and 400 mg/kg BW did not cause death or behavioral changes (nerves, seizures, ataxia) in healthy Wistar rats.<sup>9</sup> Administration of 2 ml cinnamon with a dose of 20 mg/day to HFD-rats caused a significant decrease in fasting blood glucose, insulin, and HbA1c.<sup>10</sup> Ethanol extract of *C. xanthorrhiza* Roxb with doses of 300, 2000, and 5000 mg/kg BW gave no side effects or toxic effects, and did not cause death in Swiss female mice.<sup>11</sup>

The use of four medicinal plants in the formula was to provide a better synergistic effect compared to the administration of only one herb. Many have studied the toxicity and anti- hyperglycemic

activity of each plant, but data on the efficacy in combination form is not available yet. Therefore, a study needs to be conducted to investigate the effects of combined infusion of *sambiloto*, *salam*, *kayu manis*, and *temulawak* on blood glucose, cholesterol, and triglyceride levels in Sprague Dawley (SD) rats.

## METHODS

This was an experimental study using healthy, white, male and female SD rats (*Rattus norvegicus*), aged 2-3 months. The rats were obtained from the Experimental Animal Care Unit of Universitas Gadjah Mada, Indonesia. The rats were divided into 3 groups, treatment, negative control, and positive control groups. Each group consisted of 6 white rats, males and females. The doses of *A. paniculata* and *S. polyanthum* were based on previous preclinical trials. The additional components, *C. Xanthorrhiza* and *C. Zeylanicum*, were based on empirical doses, which were converted to rat doses (multiplied by an extrapolation factor of 0.0182).

Before the study began, the rats were acclimatized for 7 days, housed in the experimental pharmacology laboratory of Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC), given drink and fed with standard pellets *ad libitum*. For inducing hyperglycemia in the rats, the treatment and positive control groups were administered HFD, a mixture of fructose (36%), egg yolk (20%), and standard pellet (44%) for 70 consecutive days. Negative control group was given standard pellets only.

Measurement of blood glucose levels as well as total cholesterol and triglycerid levels was conducted respectively on days 0, 20, 40, 60, and 70. On day 71, the hyperglycemic treatment group was given orally administered combined infusion formula, consisting of *S. polyanthum*, *A. Paniculata*(dose?or %), *C. Zeylanicum*(dose?or %),, and *C. Xanthorrhiza*(dose?or %),, at a dose of 491,4 mg/200 g BW for 7 consecutive days. The positive control group was given metformin (45 mg/kg BW) for the next 7 days. On day 77, blood was taken via retro orbital venous plexus and centrifuged. The levels of blood glucose, total cholesterol, and triglyceride were determined. At the end of study, the rats were sacrificed and the pancreas isolated. Histopathological preparations were made and examined microscopically, comparing the treatment group to the control group. The results was analyzed

using paired and unpaired t-test. This study received ethical approval from the Ethical Committee of National Institute Health Research and Development Center.

**RESULTS**

The administration of HFD for 70 consecutive days led to significantly increased glucose level as well as total cholesterol and triglyceride levels in the rats. The control group showed no significant changes in the levels of glucose, total cholesterol and triglyceride ( $p > 0.05$ ) as shown in Table 1.

The administration of combined medicinal plants infusion, which consisted of *S. polyanthum*, *A. paniculata*, *C. zeylanicum*, and *C. xanthorrhiza* at a dose of 491.4 mg/200 g BW significantly decreased the glucose level of treatment SD rat group ( $p < 0.05$ ). The total cholesterol and triglyceride levels of treatment group were also reduced. The

administration of metformin at a dose of 45 mg/kg BW decreased both glucose and total cholesterol levels (Table 2).

The rat body weights (grams) were periodically measured once a week. The change of body weight changes of both treatment and metformin groups showed no significant difference compared to the change of body weight changes of the control group as shown in table 3.

The average number of abnormal cells in pancreas in the control, treatment, and metformin groups were 23.0; 25.5 and 45.5 from 100 cells respectively (Figure 1). It can be concluded that the administration of the infusion formula at a dose of 491.4 mg/200 g BW for 70 consecutive days shows no significant difference between the abnormal cell number of the control and the treatment groups ( $P = 0.387$ ). Therefore, the administration of the infusion formula was safe for the pancreas of SD rats.

Table 1. Glucose, total cholesterol, and triglyceride levels caused by hyperglycemic induction after 70 days HFD administration

Parameters	Day/p value	Groups		
		Control	Infusion	Metformin
Glucose	0	116.83±10.028	100.33±5.279	116.5±13.096
	70	128.83±4.119	143.33±11.827	142.67±11.518
	p	0.058	0.000	0.016
Total cholesterol	0	52.17±8.727	48.50±11.589	53.33±7.421
	70	50.00±8.438	101.67±8.756	91.83±14.063
	p	0.671	0.001	0.005
Triglyceride	0	120.83±35.216	119.67±63.481	146.17±18.203
	70	98.83±17.600	460.33±163.968	293.00±182.238
	p	0.279	0.006	0.102

Table 2. Effects of combined infusion administration on glucose, total cholesterol and triglyceride levels in experimental induced hyperglycemic rats

Parameter	Day/p value	Groups		
		Control	Infusion	Metformin
Glucose	70	128.83±4.119	143.33±11.827	142.67±11.518
	77	118.83±13.934	90.17±7.985	91.50±12.029
	p	0.151	0.000	0.001
Total cholesterol	70	50.00±8.438	101.67±8.756	91.83±14.063
	77	57.67±8.869	81.83±10.088	68.17±9.432
	p	0.306	0.003	0.021
Triglyceride	70	98.83±17.600	460.33±163.968	293.00±182.238
	77	84.17±23.727	95.33±10.948	100.17±25.097
	p	0.043	0.003	0.051

Table 3. Body of weight of rats in grams

Groups	Weight (grams) in Week							W <sub>12</sub> -W <sub>0</sub>	p (95%)
	0	2	4	6	8	10	12		
Control	197.7	215.0	226.8	229.3	192.7	263.3	254.2	56.5	
Treatment	191.0	210.6	214.3	225.8	208.3	252.6	249.2	58.2	0.792
Metformin (grams)	186.9	204.3	210.9	222.7	216.0	242.0	237.9	51.0	0.289

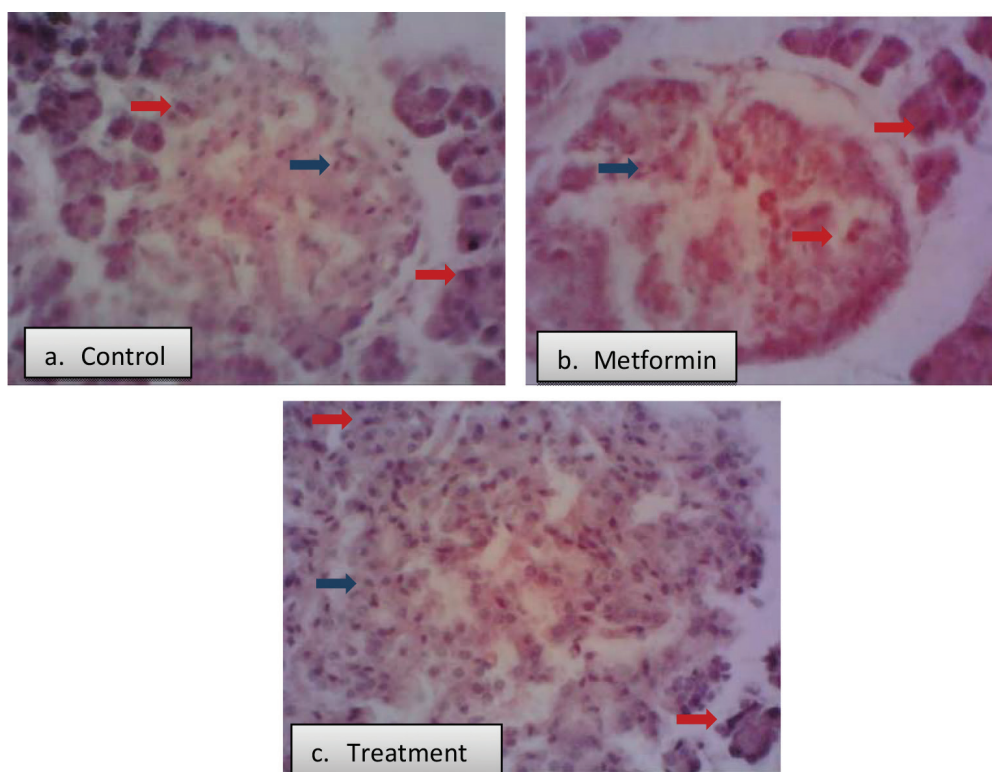


Figure 1. Pancreatic histopathology of control (a), metformin (b) and treatment (c) groups

Note: ➔ abnormal cell (in pancreas and surrounding tissues)  
➔ normal cell

**DISCUSSION**

This was an experimental pharmacological study using white Sprague-Dawley (SD) rats (*Rattus norvegicus*). The limitation of this study was bias caused by the use of laboratory animals in which glucose levels, specified as a parameter, could be influenced by several factors, such as stress and other environmental factors.

Hyperglycemia in rats was induced by administering a high fructose diet (HFD), a mixture of 36% fructose, 20% egg yolk and 44% standard pellets, for 70 days. Measurement of rat blood glucose levels was conducted on days 0, 20, 40, 50, 70, and 77???(explained). The results (table 1) showed

that the administration of HFD cause significant changes in blood glucose levels of rats on day 70. It has been postulated that increasing consumption of fructose may be a contributory factor in the development of obesity and the accompanying metabolic abnormalities. Most studies supporting these hypotheses, suggested that consumption of high amounts of fructose may stimulate lipogenesis and thus alter lipid metabolism and increase body weight.<sup>12</sup> Several studies regarding the relationship between HFD with elevated levels of plasma glucose, insulin, and triglycerides have been made. Twelve week administration of 10% w/v fructose solution improved blood glucose and triglyceride levels in male Wistar rats. In another study, supplementation of

10% w/v HFD for 4 weeks induced insulin resistance on SD male rats.<sup>13</sup> In this study, HFD also caused a significant increase in cholesterol and total triglyceride. Previously reported that the administration of 10% w/v fructose solution for 12 weeks increased triglycerides in male Wistar rats. Fructose can be absorbed rapidly and is metabolized in liver.<sup>13</sup>

The administration of combined infusion was found to have a positive antihyperglycemic effect by decreasing glucose level in the rats. This could be caused by a specific compound contained in *A. paniculata* and *S. polyanthum*. While *C. Xanthorrhiza* and *C. zeylanicum* were used as additional supportive effect. *A. paniculata* contains andrographolid which can increase the use of muscle glucose in streptozotocin induced diabetic rats through stimulation of glutathion-4 transporter resulting in reduced plasma glucose levels. Andrographolid also increases the utilization of glucose by overexpressing mRNA and glutathion-4 protein levels.<sup>14</sup>

There was no significant body weight (week 0 - week 12) difference between treatment and control groups. This indicated that the administration of high fructose diet for 70 consecutive days resulted in the same body weight changes in the treatment and control groups. In a previous research, a diet of 8% high fructose (corn syrup) in male SD rats with a 12-hour access to consumption, caused a significant increase in body weight of rats on week 8.<sup>15</sup> While the consumption of the same diet for 6 months led to a significant increase in body weight, fat, and triglyceride levels compared to the control group. The increase of body weight was a result of daily HFD consumption.<sup>16</sup>

In order to determine inflammatory effects on the cell nucleus, observations of abnormal cells were made on every 100 cells, by counting the number of inflammatory mononuclear cells in the islet Langerhans and surrounding cells with a magnification of 400x. At this magnification, the pancreatic cell could be distinguished from surrounding tissues. There was no significant difference of the abnormal cell number between the control and treatment rats groups. This meant that the administration of the combined infusion did not cause negative histopathologically effect (obstruction) on the rat pancreas. Abnormal cells of pancreas can appear as a result of direct toxic effects of certain chemicals, autoimun reaction, or viral infection. There are many levels of insulinitis i.e perinsulinitis (limphocyte infiltrating surrounding tissues of the pancreas), medium insulinitis (more

than 50% of the islet of Langerhans is infiltrated by limphocytes), hard insulinitis (more than 50% of the islet of Langerhans is infiltrated by limphocytes) and end stage islet in which necrosis is found in the entire of islets of Langerhans.

In conclusion the administration of the combined infusion formula at a dose of 491.4 mg/200 g BW for 7 days decreased blood glucose, cholesterol and triglyceride levels of hyperglycemic rats and was considered safe for the pancreas.

### Acknowledgment

A special thanks to Prof. Abdul Rohman for reviewing this article. This study was funded by MPTMRDC, National Institute of Health Research and Development, Ministry of Health Republic of Indonesia.

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