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Antidiabetes Activity of Herbal Product Containing *Phyllanthus niruri* and *Zingiber americans*

(Aktiviti Antidiabetes Produk Herba yang Mengandungi *Phyllanthus niruri* dan *Zingiber americans*)

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ABSTRACT

The diabetes mellitus is one of the chronic diseases that has high prevalence rates in the world. It is defined as a state of hyperglycemia which usually accompanied by increasing blood glucose level. The combination of Phyllanthus niruri *herbs and* Zingiber americans *rhizome has been produced as herbal medicine for the treatment of diabetes. The efficacy of combination of these plants is still based on empirical data. This research aimed to prove the antidiabetes activity of herbal medicine product containing* P. niruri *and* Z. americans in vivo. *Antidiabetes activity was tested using oral glucose tolerance and alloxan induced methods. The blood glucose levels were measured in each experiment. In addition, the plasma triglyceride and total cholesterol levels were also measured. The results showed that the decrease of blood glucose levels on oral glucose tolerance method were 40.98, 44.84, and 47.02% at doses of 90, 135, and 203 mg/kg BW, respectively ($p < 0.05$) compared to control. While the decrease in blood glucose levels in alloxan induced method were 33.05, 38.82, and 41.79% at dose 90, 135, and 203 mg/kg BW, respectively ($p < 0.05$) compared to control. The extract of dose 203 mg/kg BW appeared to be the most potent to decrease blood glucose levels in both methods, but did not show suppression activity of triglycerides and total cholesterol plasma. Suppression activities of serum triglycerides and total cholesterol were shown after treatment with the extract dose 90 and 135 mg/kg BW, which were significantly different to control ($p < 0.05$). The herbal product contains flavonoid, phenol and tannin, which total of each were 0.15, 0.16, and 1.85% w/w, respectively.*

Keywords: Diabetes; herbal medicines; jamu; Phyllanthus niruri*;* Zingiber americans

ABSTRAK

Penyakit kencing manis ialah salah satu penyakit kronik yang umum di dunia. Penyakit ini didefinisikan sebagai keadaan hiperglikemik yang diikuti dengan tingginya kadar gula dalam darah. Kombinasi tanaman herba Phyllanthud niruri *dan* Zingiber americans *telah dijadikan sebagai produk ubat herba untuk mengubati penyakit* diabetes*. Kesan daripada gabungan dua tanaman ini masih berdasarkan kepada data empirik. Kajian ini dijalankan untuk membuktikan khasiat anti-diabetes daripada salah satu produk ubatan herba yang mengandungi* P. niruri *dan* Z. americans secara in vivo*. Kajian ke atas anti-diabetes ini dijalankan menggunakan kaedah induksi aloksan. Kandungan gula dalam darah untuk setiap percubaan dihitung. Selain itu, kadar trigliserids dan kolestrol juga diukur. Hasil menunjukkan bahawa kadar gula dalam darah dapat diturunkan dengan kaedah toleransi glukosa sebanyak 40.98, 44.84 dan 47.02% dengan dos 90, 135 dan 203 mg/kg BW ($p < 0.05$) berbanding kawalan. Penurunan dengan kaedah induksi aloksan pula adalah 33.05, 38.82 dan 42.79% pada dos 90, 135 dan 203 mg/kg BW ($p < 0.05$) berbanding kawalan. Ekstrak yang mengandungi dos 203 mg/kg BW menunjukkan penurunan kadar gula dalam darah paling tinggi dalam kedua-dua kaedah yang digunakan, namun tidak menunjukkan aktiviti penindasan trigliserids dan jumlah plasma kolestrol. Aktiviti penindasan serum trigliserids dan jumlah kolesterol dilihat selepas rawatan dengan ekstrak dos 90 dan 135 mg/kg BW, berbanding kawalan ($p< 0.05$). Produk herba ini mengandungi flavonoid, fenol dan tanin dengan jumlah sebanyak 0.15, 0.16 dan 1.85% w/w.*

Kata kunci: Jamu; kencing manis; Phyllanthus niruri; *ubat herba;* Zingiber americans

INTRODUCTION

Herbal medicines have been used by community to treat diseases and to maintain health since long ago. They have been developed from traditional way into modern processing and produced as herbal medicines by household into big industries. The development of herbal medicines product should be followed by the good quality production to prove the efficacy, the quality and standardization (Elfahmi et al. 2014). Herbal medicine produced by small enterprise has been marketed for antidiabetes. Diabetes

melitus (DM) is a chronical disease with high prevalence in the world. In Indonesia, the diabetes patient tends to rise every year where in 2000, there were 8.4 million people with diabetes and is predicted to rise around 21.3 million in 2030 (Wild et al. 2004). In 2019, the diabetes patients reach 463 million people (IDF 2019; WHO 2019). This disease is defined as hyperglycaemic with abnormalities of carbohydrate, protein, and lipid metabolism as well as abnormality of production and activity of insulin (Shan et al. 2006). The use of antidiabetes drugs tend to increase since the rising of diabetes cases. Many medicinal plants have been reported to have antidiabetes activity. Meniran or Dukung anak (*Phyllanthus niruri* L.) belonging to family Phyllanthaceae is a medicinal plant that has been used traditionally for treat diabetes (Calixto et al. 1998; Nwanjo et al. 2017). Empirically, this plant has been also used mostly for the remedy of fever, inflammation, malaria, lithiasis, gonorrhea, hepatitis, genitourinary, hypertension, cancer, skin, digestive, hepatic, and respiratory disorders (Jantan et al. 2019; Sarin et al. 2014). The pharmacological activities of this plant were intensively studied to prove its traditional use. Methanol extract of this plant showed the highest inhibitory effect against non-alcohol-fatty liver disease through reduction of several parameters such as hepatomegaly, visceral fat weight, prevented fibrosis, serum total cholesterol, low-density lipoprotein, and free fatty acids. It also inhibited the activity of enzymes related to diabetes such as α-glucosidase and pancreatic lipase enzymes (Al Zarzour et al. 2018, 2017). The methanol extract was reported to have anti-inflammatory activity and protect the ulcer through the regeneration of mucosal layer and substantial prevention of the formation of hemorrhage and edema (Mostofa et al. 2017). While the aqueous extract reduced the amount of lipid peroxidation product, and inhibited the enzymes related to the oxidative stress such as superoxide dismutase, catalase, and glutathione peroxidase in the kidney of diabetic rats (Giribabu et al. 2018). *P. niruri* has been reported to be potential developed as an anticancer since it inhibits cancer cell *in vitro* (Junior et al. 2012; Tang 2013; Zheng et al. 2016). In addition, the pharmacological effects of this plant as anti-parasite, anti-bacterial, anti-virus, cardiovascular protective, antioxidant, anti-aging and skin protecting have been also reported (Nisar et al. 2018; Sarin et al. 2014). Only few reports have been published on the antidiabetes activity of *P. niruri in vivo* (Bavarva & Narasimhacharya 2007). One of them reported that *P. niruri* extract decreased the serum glucose level of type 2 diabetes in Sprague-Dawley rats induced by streptozotocin (Mediani et al. 2016). Some chemical compounds from several secondary metabolites group have been isolated from this plant. It contains flavonoid group such as quercetin and rutin; alkaloids: allonorsecurinine, ent-norsecurinine, and nirurine; phenolic such as gallotannins corilagin and geraniin. The most abundant compound found in *P. niruri* is lignin group such as phyllantin, hypophillantin, cubebin, nirtetarlin, and tetralin (Jantan et al. 2019; Nisar 2018). Two lignan compounds have been found in cell suspension cultures of *P. niruri* that has not been found in the original plant (Elfahmi et al. 2014). Lempuyang pahit (*Zingiber americans* Bl.) belong to family Zingiberaceae has been also used traditionally for the treatment of diabetes, stomach disease, and reducing blood level (Al-Amin et al. 2006; Sukari et al. 2008). The scientific reports on pharmacology study of this plants are still limited. Combination of meniran herb and lempuyang pahit rhizome has been produced and marketed as herbal medicine belong to *jamu* by a small enterprise for the treatment of diabetes. Nowadays, the evidence for the efficacy of this herbal medicine product is still based on empirical data. Therefore, there is no scientific evidence for the combination of two plants for the treatment of diabetes. The research was conducted to prove the claimed efficacy as antidiabetes. Antidiabes activity was studied using glucose tolerance and alloxan induced methods. In addition, the phytochemical study was also conducted for herbal medicine product to know the phytochemical profile for standardization.

Materials and Methods

Plant and chemicals

The sample used was herbal medicine product containing extract of *Phyllanthus niruri* L. and *Zingiber americans* BI, metformin (PT. Kalbe Farma, Jakarta, Indonesia), alloxan monohydrate (Sigma Aldrich, Darmstaad, Germany), amyl alcohol, magnesium, chloroform, ammonia 25%, Dragendorff, and Mayer reagent, $FeCl_3$, CMC-Na, Al_2Cl_3, sodium acetate, sodium bicarbonate, methanol, ethanol, toluene, and acetone (all reagent and solvents were analytical grades).

Phytochemical screening and standardization

Phytochemical screening was carried out to identify the secondary metabolites groups in the extract of herbal medicines product which contain *P. niruri* and *Z. americans*. The secondary metabolites measured were alkaloid, flavonoid, tannin, saponin, quinone, and steroid/triterpenoid. Quantitative measurement of total flavonoid was done by colorimetric method (Chang 2002). While the total phenolic compound was measured by Spectrophotometer UV. Several parameters for standardization such as total ash content, water content, water and ethanol extractable matter and TLC profile were measured according to Indonesian Herbal Pharmacopoea (IMH 2008).

ANTIDIABETES ACTIVITY USING GLUCOSE TOLERANCE METHOD

Male mouse Swiss Webster age 2-3 months were used as animal model and divided into several groups. Animals were fed and adapted at the lab according to standard procedures. Samples were prepared with dissolving of the extract from capsule of herbal medicines product with tragacanth 0.5%. Antidiabes activity was carried out using glucose tolerance method and alloxan induced method (Shan et al. 2006). For glucose tolerance method, mouse was fasted for 10-12 h before induction, then randomly divided into 5 groups consist 5 mice of each group. Fasting blood glucose level were measured (T_0). Control group was given CMC Na 0.5%, standard group was given metformin 195 mg/kg bb, treated groups were given the extract with dose 90, 135, and 203 mg/kg BW. Thirty minutes after treatment, each group was given glucose solution with concentration 3 g/kg BW per oral. Blood glucose levels were measured at 30, 60, 90, and 120 min after glucose treatment.

ANTIDIABETES ACTIVITY USING ALLOXAN INDUCED METHOD

For alloxan induced method, mouse was fasted for 10-12 h. Before induction, fasting blood glucose levels were measured so called H_0. Mouse were induced with alloxan dose 57 mg/kg BW i.v. On day 7 after induction (H_7), blood glucose levels were measured. Samples were given to mouse with the blood glucose level higher than 225 mg/dL. Diabetic mouse was randomly divided into five groups. Control group was given CMC-Na 0.5%. Standard group was given metformin 195 mg/kg BW. Three treated groups were given extract sample with three different concentrations which were 90, 135, and 203 mg/kg BW, respectively. Prior to treatment, samples were suspended in CMC-Na 0.5% solution. The treatment was done during 21 days. Blood glucose levels of all mouse groups were measured on day 8, 10, 14, 21 and 28 using glucose strip test and glucometer GlucoDr™. Data were statistically analyzed using ANOVA method.

CHOLESTEROL AND TRIGLYCERIDE ANALYSIS

In addition, total cholesterol and triglyceride levels were measured before induction (H_0), and after induction (H_7). Cholesterol total was measured using enzymatic colorimeter method. Three type of samples were prepared, which were blank, standard, and treated solution. Blank solution consists of total cholesterol reagent. Standard solution consists of 1 mL of reagent and 10 µL of standard solution. Treated samples consist of 1 mL reagent and 10 µL of tested animal serum. All solution was incubated at room temperature (18-30 °C). Absorbance of all solutions were measured at wavelength of 546 nm. Total triglyceride levels were measured using GPO enzymatic colorimeter method. Three types of solution as mentioned with different reagent were prepared, followed by the same procedure with determination of total cholesterol level.

RESULTS AND DISCUSSION

The herbal product which contains *P. niruri* and *Z. americans* produced by small enterprise was registered in Indonesian FDA. This herbal medicine product was classified as *jamu* or traditional medicine since the efficacy claim is based on empirical data. There is no scientific study to prove the claim yet. Through the community services supported by Indonesian Ministry of Higher Education and with collaboration with the producer, the *in vivo* antidiabtes study of the product was carried out. Phytochemical study and quantitative measurement of the extract sample, showed that the product contain flavonoid, phenol, and tannin, with total of each were 0.15, 0.16, and

TABLE 1. Standardization parameter of extract sample was compared to the standard according to Indonesian herbal pharmacopoeia (FHI)

Parameter	Extract (%)	*Phyllanthus niruri* extract (FHI in%)
Water content	3.98*	< 17
Total ash content	1.04	< 3.5
Lost on drying	6.15	-
Water extractable matter	67.58	-
Ethanol extractable matter	6.69	-
Total flavonoid	0.15	3.2
Total phenol content	0.16	-
Total tannin content	1.85	-

1.85% w/w, respectively. For standardization process, some parameters are shown in Table 1, indicated that the extract sample fulfilled the standard of Indonesia herbal pharmacopoeia for water and ash contents, however, the flavonoid total was below standard. Others parameter were not included in FHI for extract monograph, they are included in the crude dried plant material. These parameters indicated the quality of extract (IMH 2008).

Further analysis of chemical components of extract sample using thin layer chromatography (TLC) showed the chromatogram profile as Figure 1. Almost all bands showed by the *P. niruri* and *Z. americans* were exist in the extract sample. At least 5 of 9 bands which was shown in extract samples have similar Rf value as shown by *P. niruri* extract. While 4 other bands of sample extract have similar Rf value as shown by *Z. officinale* extract. These results confirmed that the herbal medicine product contains these two plant extracts. In addition, after visualization using citroborate, a specific spray reagent for flavonoid, also confirmed the standard of extract sample. One of the bands was a quercetin compound.

Antidiabes test using glucose tolerance method was done for normal male mouse with blood glucose level ranging 96-107 mg/dL. The value complies with the value of normal value published before which is below 199 mg/dL (Fajardo et al. 2014). The study was carried out to determine whether the extract sample inhibit the increase of blood glucose level and recover it into normal after the increase caused by glucose induction. The blood glucose level increased for all animal groups, 30 min after glucose intake (T_{60}), The treated groups showed the lower increase of blood glucose level compared to control group. After T_{60}, the reducing of blood glucose level was significant. Sixty minutes after treatment (T_{90}), there was significant different of blood glucose level for control, the samples of dose 90, 135, and 203 mg/kgBW compared to T_{60} (Figure 2). Measurement after T_{60}, each treated group showed the reducing of blood glucose level and significantly different to control group ($p<0.05$). Metformin as a standard showed the highest reducing of glucose blood level. Metformin reduced the blood glucose level with the mechanism to increase the insulin sensitivity in liver and peripheral tissue. It reduces the production of glucose by liver and increase the glucose intake by peripheral tissue (AHFS 2008). The different of reducing level in T_{60}-T_{120} and T_{60}-T_{150} for treated groups is not significant to standard metformin group. The reducing of blood glucose levels after 120 min treatment with dose 90, 135, and 203 mg/kg BW were 40.98, 44.84, and 47.02%, respectively. The group treated with dose 203 mg/kg BW gave the reducing level similar to standard metformin and appear as the dose with highest level of reducing (Figure 3). It assumed that the sample was not only able to suppress the increase of blood glucose level, but also to reduce it.

For alloxan induced method, mouse was induced using alloxan monohydrate. Alloxan was given with dose 57 mg/kg BW i.v. Alloxan has the diabetogenic effect to β-pancreas cell through some mechanism such as inhibition of glucokinase activity, production of free radical and disturb the homeostasis of intracellular calcium ion (Szkudelski 2001). The time needed to provide diabetic animal with stable blood glucose level above 225 mg/dL was normally reached after 3-7 days. On day 7 after induction, blood glucose level of mouse was ranging 312-455 mg/dL. This range was big enough, since the alloxan effect is influenced by nutrition status and age of animal model (Szkudelski 2001). Diabetic animal model for this study was moderate-high model. Diabetic condition was provided due to the alloxan activity toward β-pancreas caused insulin deficiency. Profile of glucose level for day 7-28 is similar between standard and treated groups. Day 14, standard and treated groups with dose 203 mg/kg BB showed the statistically significant different ($p<0.05$) on blood glucose level compared to day 7. While other treated group dose (90 and 135 mg/kg BW) showed the different blood glucose level on day 21. This was assumed that the dose 203 mg/kg BW gave the reducing blood glucose level faster than others. On this day, metformin and all three treated groups showed the reducing blood glucose level which were statistically significantly different to the control (Figure 4).

One day after treatment (H_8), standard group showed the highest reducing of blood glucose level compared to all other groups. On day 10 and 14, the highest percentage was shown by treated group with dose 90 mg/kg BW. While on day 21, the highest reducing of blood glucose level was shown by treated group on dose of 135 and 203 mg/kg BW and have almost similar percentage. On day 28, the highest percentage was shown by treated group on dose of 203 mg/kg BW. The percentage of reducing was dose dependent on this day. On day 1 after induction, treated group on dose 203 mg/kg BW showed the increase of blood glucose level compared to previous days. Single treatment of this dose did not show the decreasing of blood glucose level yet. The reducing activity increased after continuously treatment. At the end of treatment, on day 28, the highest reducing was given by treated group with dose 203 mg/kg BW. Percentage of reducing blood glucose level on day 28 for standard, treated group with dose 90, 135, and 203 mg/kg BW were 33.05, 38.82, 41.79, and 44.42% (Figure 5). This showed that the dose 203 mg/kg BW gave the optimal activity for reducing of blood glucose level.

For diabetes patients, it is often accompanied with the increase of serum triglyceride and cholesterol levels. This is caused by the mechanism of insulin in inhibition of lipase activity. Lipase break the lipids so called lipolysis which convert triglyceride into glycerol and fatty acids.

The fatty acids are re-synthesized into triglyceride. Alloxan cause the defect of β-pancreas leading to insulin deficiency. As the compensation, lipolysis process is not inhibited leading to the accumulation of fatty acids. This cause the increase of re-synthesis of triglyceride and other lipoprotein including cholesterol. The highest blood glucose level related to the increase the triglyceride level. Through the biochemical process, glucose is converted into acetyl-CoA, which is converted further into fatty acids. Fatty acids from lipogenesis as well as from the food intake will be converted into triglyceride with the esterification.

Triglyceride (TG) and total cholesterol (CL) profile of mouse serum on day after induction with alloxan showed the increase of both for all groups. The increase of TG was ranging 143-185 mg/dL (Figure 6), while the increase of CL was ranging 137-178 mg/dL (Figure 7). According the European Atherosclerosis Society, hypercholesterolemia and hypertriglyceridemia were shown by plasma CL and TG level above >200 mg/dL. The TG and CL of the animal model in this study were not classified as hypercholesterolemia and hypertriglyceridemia but have the risk of them. The TG and CL level showed in Figures 6 and 7 have almost similar profile, where the decrease of TG and CL level was shown by standard group which was statistically significant compare to the control ($p<0.05$). Treated groups on dose of 90 and 135 mg/kg BW suppressed the increase of TG and CL level, while treated group on dose of 203 mg/kg BW did not show suppressed the decrease of TG and C level. The decrease activity on TG and CL was not shown by all three treated groups since there were not statistically different to standard group ($p<0.05$).

Metformin has the mechanism to increase activity of insulin in liver, muscle, and fat. Insulin has a role in metabolism of glucose and lipid. Insulin suppress the production of liver glucose, breaking of glycogen. In fat tissue, glucose is converted into free fatty acids and stored in a form of triglyceride. Insulin protect the breaking of triglyceride into free fatty acids, the form that can be transported into other tissues to be used. Insulin decrease free fatty acids in circulation and stimulant the storage of triglyceride and cholesterol and adipocyte (Setter & Campbell 2000). Therefore, the metformin group showed the decrease of blood glucose level followed by the decrease of serum lipid level.

P. niruri has been studied its antidiabetes, hipolipidemia, and antioxidant activities on diabetic rat. Hypogycaemic effect is probably due to the insulin activity or stimulant of insulin effect (Bavarva 2007). Zerumbone is a main component of *Z. americans* that has been studied its antioxidant and hepatoprotective activities (Fakurazi 2008). The antioxidant activity could protect β-pancreatic cell damage from a reactive- oxygen species (ROS) which is radical specied produced by alloxan.

The sample extracts only showed the suppression of the increase of TG and CL level on dose of 90 and 135 mg/kg BW. They did not show the decrease of TG and CL level. This is probably caused by the effect on the inhibition of resistance of TG and CL and did not influence toward the storage of TG and CL into adipocyte tissues, so that the TG and CL level in plasma was not changed after treatment.

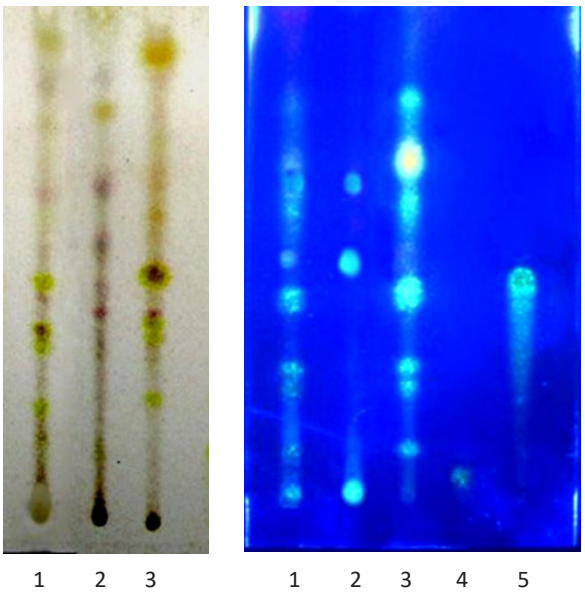

FIGURE 1. TLC chromatogram of sample extract (1), *Phyllanthus niruri* extract (2), *Zingiber americans* extract (3), quercetrin (4) and quercetin (5), visualization with spray reagent H2SO4 10% in methanol (left), citroborate (right), silica gel plate GF254, toluene-acetone (1:1) as mobile phase

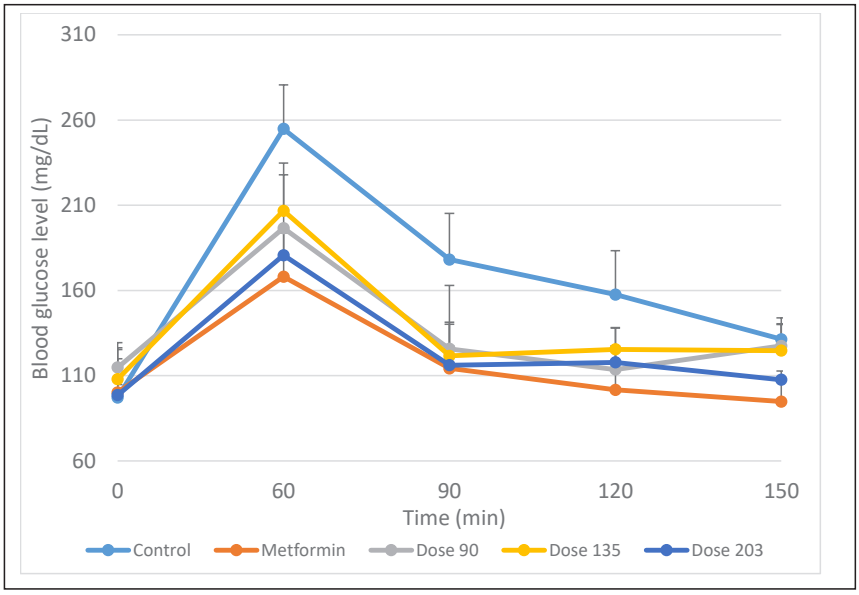

FIGURE 2. Blood glucose level profile using glucose tolerance method. Dose are in mg/kg BW, Individual values expressed as means ± standard deviation are averages of three independent experiments

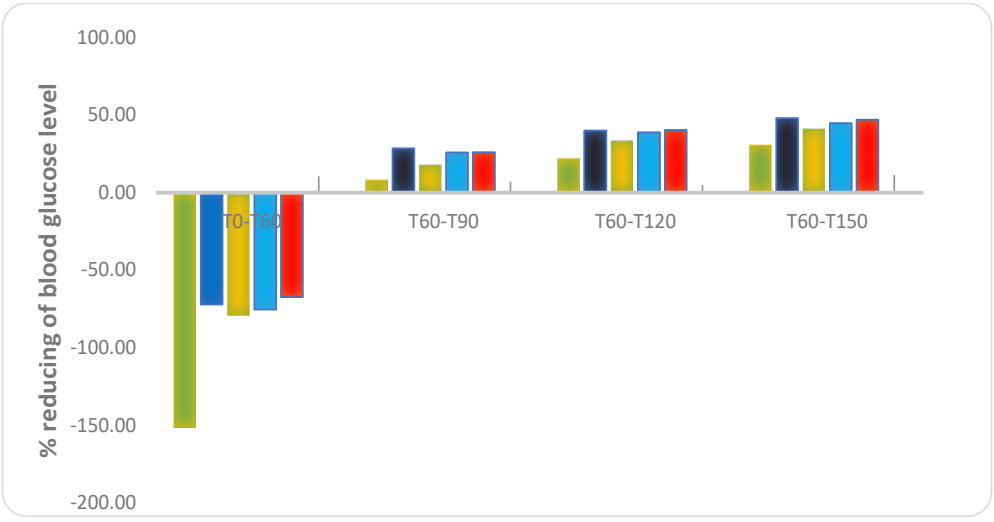

FIGURE 3. Percentage of the reducing of blood glucose level profile using glucose tolerance method. T60-T0 = Day 60 compare to day 0, T60-T90, T60-T120, and T60-T150 = day 90, 120 and 150 respectively compare to day 60. Individual values expressed as means ± standard deviation are averages of three independent experiments

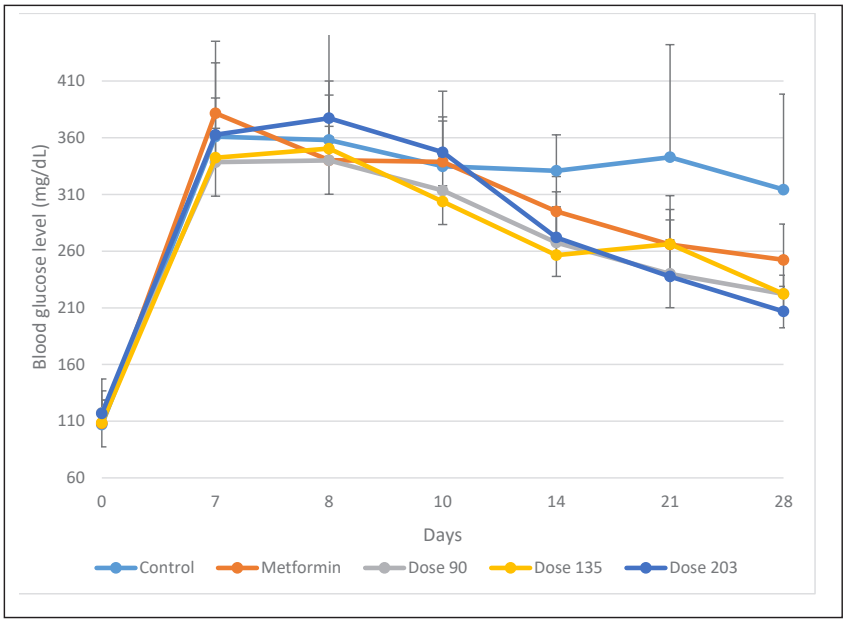

FIGURE 4. Blood glucose level profile using alloxan induced method. Dose are in mg/kgBW, Individual values expressed as means ± standard deviation are averages of three independent experiments

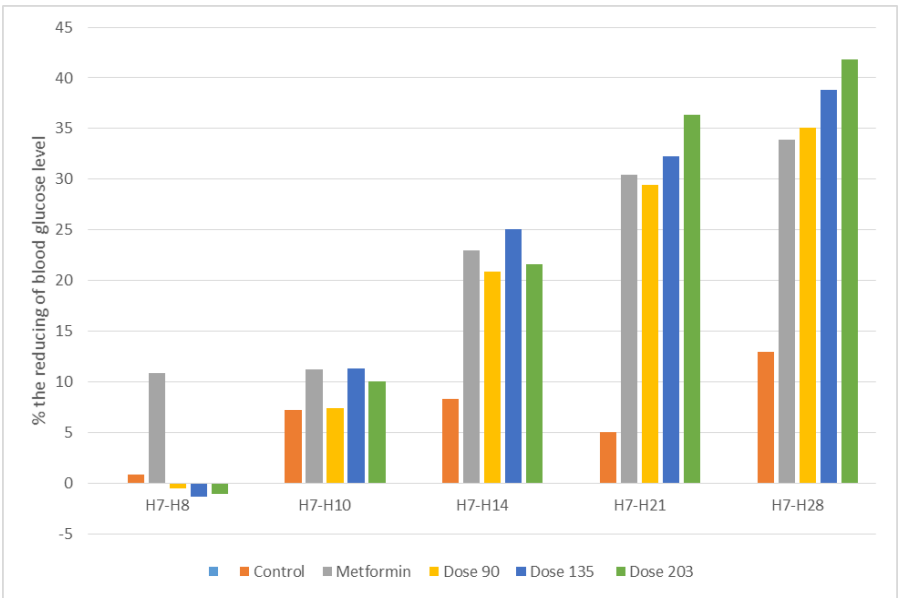

FIGURE 5. Percentage of the reducing of blood glucose level profile using alloxan induced method. Day 10, 14, 21 and 28 compare to day 7. Individual values expressed as means ± standard deviation are averages of three independent experiments

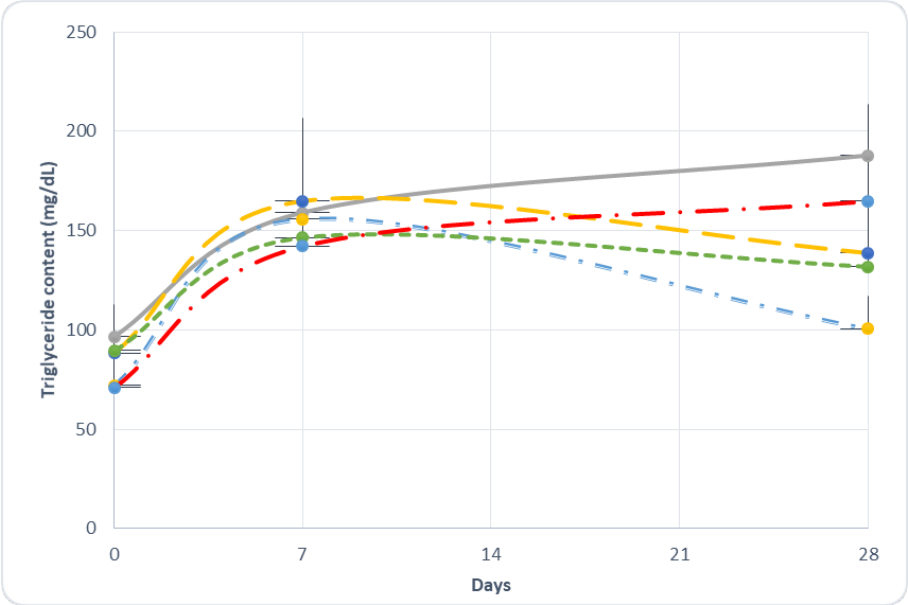

FIGURE 6. Triglyceride profile of all group using alloxan induced method. . Individual values expressed as means ± standard deviation are averages of three independent experiments. = control, = standard, = dose 90 mg/kg BW, = dose 135 mg/kg BW, = dose 203 mg/kg BW

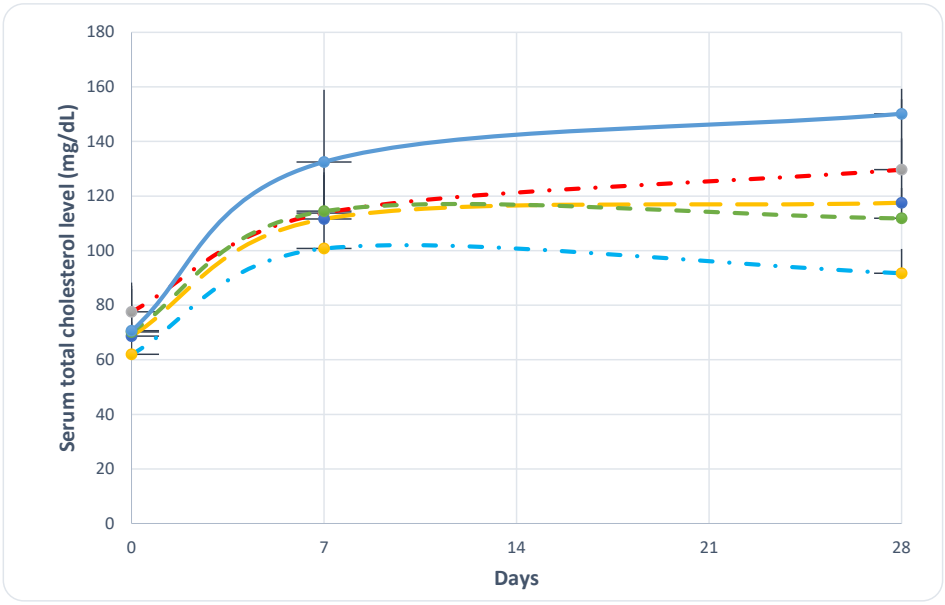

FIGURE 7. Total cholesterol profile of all group using alloxan induced method. Individual values expressed as means ± standard deviation are averages of three independent experiments. = control, = standard, = dose 90 mg/kg BW, = dose 135 mg/kg BW, = dose 203 mg/kg BW

Conclusion

Herbal product which produced from medicinal plants *Phyllanthus niruri* and *Zingiber americana* contains flavonoid, phenol, and tannin with concentration of 0.15, 0.16, and 1.85%, respectively. The extract with all doses showed the reducing of blood glucose level of animal model. The optimum reducing of glucose blood level was given by the extract dose 203 mg/kg BW. The reducing cholesterol and triglyceride level were given by extract dose 90 and 135 mg/kg BW.

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