PIC
Pharmacy International Conference

Certificate of Appreciation

UNIVERSITAS MUHAMMADIYAH PURWOKERTO

This Certificate is Honorably Presented To

Ai Sri Kosnayani

For the excellent Oral Presentation at
The 1st University of Muhammadiyah Purwokerto Pharmacy International Conference 2015 (UMP-PIC 2015)
"Collaborative approach to improve research and management of chronic diseases"
Held at the Ballroom of Horizon Hotel, 5th – 6th June 2015, Purwokerto, Indonesia

Githa Fungie Galistiani S.Farm.,M.Sc.,Apt
The 1st UMP-PIC Chair

Dr. Nunuk Aries Nurulita S.Si.,M.Si.,Apt
College Dean of Pharmacy

3 SKP (Credits Participation Unit) No.117/SK-SKP/PP.IAI/IV/2015

Faculty of Pharmacy
Muhammadiyah University of Purwokerto
Jl. Raya Dukuh Waluh Kembaran Purwokerto PO BOX 202
Telp. (0281) 636751, 630463, 634424
Website : http//farmasi.ump.ac.id
Email : farmasiump@gmail.com

ISBN 978-602-73538-0-0

PROCEEDING

The 1st University of Muhammadiyah Purwokerto - Pharmacy Internasional Conference (UMP-PIC)

PIC
Pharmacy International Conference

PROCEEDING

The 1st University of Muhammadiyah Purwokerto - Pharmacy Internasional Conference (UMP-PIC)

5-6 June 2015

"Colaboration Approach to Improve Research and Management of Chronic Diseases"

ISBN 978-602-73538-0-0

Faculty of Pharmacy

UMP

THE 1ST UNIVERSITY OF MUHAMMADIYAH PURWOKERTO – PHARMACY INTERNATIONAL CONFERENCE (UMP-PIC) PROCEEDING

5-6 JUNE 2015
Horison Hotel, Purwokerto, Central Java, INDONESIA

Editors:

Dr. Asmiyenti Djaliasrin Djalil, M.Si.
Dwi Hartanti, M. Farm., Apt.
Pri Iswati Utami, M.Si., Apt.
Susanti, M.Phil., Apt.

FACULTY OF PHARMACY
UNIVERSITY OF MUHAMMADIYAH PURWOKERTO
Purwokerto, Central Java, INDONESIA

THE 1ST UNIVERSITY OF MUHAMMADIYAH PURWOKERTO – PHARMACY INTERNATIONAL CONFERENCE (UMP-PIC) PROCEEDING

ISBN: 978-602-73538-0-0

Published by:
Faculty of Pharmacy
University of Muhammadiyah Purwokerto
Jl. Raya Dukuhwaluh, PO Box 202, Purwokerto 53182
Telp : (0281) 636751, 630463 psw 231-232
Fax : (0281) 637239
Email : farmasi@ump.ac.id
Website : www.ump.ac.id

CONTENTS

Greetings from the Dean Faculty of Pharmacy
University of Muhammadiyah Purwokerto

It is a great honour for Faculty of Pharmacy, University of Muhammadiyah Purwokerto to welcome all of invited speakers and researchers at The First University of Muhammadiyah Purwokerto – Pharmacy International Conference (UMP-PIC). Faculty of Pharmacy, UMP would like to held this confence periodicly every two year. For the first time, we have a topic, "Collaborative Approach to Improve Research and Management of Cronic Diseases". The aim of this conference is to promote the utilization and research development of cronic diseases toward patients management including prevention, therapy and health promotion.

Chronic diseases, such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are by far the leading cause of mortality in the world, representing 68% of all deaths in 2012. Out of the 38 million people who died from chronic disease, more than 40% of them (16 million) were premature deaths under age 70 years. Almost three quarters of all Chronical diseases deaths (28 million), and the majority of premature deaths (82%), occur in low- and middle-income countries. This invisible epidemic is an under-appreciated cause of poverty and hinders the economic development of many countries that is why then called non communicable diseases (NCD) (WHO, 2014).

I hope that all scientists and researchers participating in this event will present and discuss current international strategies for prevention and control of chronic noncommunicable diseases (NCD), to make participants familiar with the Finnish experiences from the North Karelia Project and to train in planning, implementation and evaluation of NCD prevention interventions: 'from theory to practice'. I am sure that this event will give advantages for all of us.

Lastly, I would like to express my deep gratitude to all participants for their contributions to the success of this event. I hope all of you will have a fruitful meeting and a pleasant stay in Purwokerto.

Dr. Nunuk Aries Nurulita, M.Si., Apt.
Dean of Faculty of Pharmacy
University of Muhammadiyah Purwokerto

Greetings from the Chairperson of the Organizing Committee
The 1st University of Muhammadiyah Purwokerto – Pharmacy International Conference (UMP-PIC)

Dear Colleagues,

The Organizing and Scientific Committee of the 1st UMP-PIC (University of Muhammadiyah Purwokerto-Pharmacy International Conference) is very pleased to announced its 1st Biannual Conference which will take place from June 5-6, 2015 at the Horison Hotel, Purwokerto, Central Java, Indonesia. The theme of this meeting is "Collaborative approach to improve research and management of chronic diseases". It will focus on the emerging role of pharmacists in chronic care. We have been able to invite experts in the field of science and cinical pharmacy to share about their experience in managing chronic diseases. Conference sessions will include plenary lectures, oral and poster presentations.

The 1st UMP-PIC invites pharmacists and pharmaceutical scientists from all over the world to delve into research and healthcare management in chronic diseases. All the organizing and scientific committee are greatly looking forward to welcoming the participants of the 1st UMP-PIC in Purwokerto, Central Java, Indonesia to experience the wonderful Indonesian hospitality. Come to the 1st UMP-PIC and gather at the most important meeting for our global network of pharmacists. We look forward to seeing you here!

Githa Fungie Galistiani, M.Sc., Apt.
Chairperson

Proceeding of The 1st University of Muhammadiyah Purwokerto-Pharmacy International Conference
5-6 June 2015, Horison Hotel Purwokerto
ISBN 978-602-73538-0-0

Effects of *Phyllanthus niruri* Linn., Metformin and Combination of Both to Body Weight, Fasting Blood Glucose, Triglyserides and HDL in Obese Rats

Ai Sri Kosnayani

Faculty of Health Sciences, Siliwangi University, Jl. Siliwangi No. 24, Tasikmalaya, East Java, Indonesia (E-mail: aisrikosnayani@unsil.ac.id)

ABSTRACT

Obesity is always relevant with insulin resistance. Insulin resistance if not treated will develop into DM or CVD. Insulin resistance can not be cured but controlled, through the management of obesity, with non-pharmacologic or pharmacologic like using metformin. Metformin can reduce weight, blood glucose, and improve the lipid profile, but it still has the disadvantage of indigestion and decrease the absorption of vitamin B12, therefore it is necessary to find an alternative form of herbal medicine. *Phyllanthus niruri* Linn. is known to be hypoglycemic, hypotensive, anti-oxidants and can control weight. This study aims to determine the effect of extracts of *P. niruri*, metformin and the combination of both to improve insulin resistance in obese rats. This is a pure experimental study. The effect of repeated administration of water extract of *P. niruri* 400 mg/kg BW, metformin 45 mg/kg BW, and the combination of both was evaluated in obese and normal Sprague dawley rats. The indicators measured were body weight, fasting blood glucose, triglycerides and HDL were measured every week. Water extract of *P. niruri*, Metformin, and a combination of both can give the effect of controlling weight gain did not differ from that of normal rat groups ($p>0.05$), lower blood glucose levels, increase HDL and lowering triglycerides with different effects ($p<0.05$). The largest effect is given by the combination of the water extract of *P. niruri* and metformin. It is concluded that the extract of *P. niruri* used with metformin was effective to improve insulin resistance in obesity rats.

Key words: insulin resistance, Lee index, metformin, obesity, *P. niruri*.

INTRODUCTION

Obesity is defined as a nutritional disorder that is characterized by the accumulation of excess fat or abnormal that could affect health. Obesity is associated with insulin resistance (Abel, 2010). Obesity and insulin resistance are always accompanied by hypertension, hypertriglyceride, and low HDL, a condition called metabolic syndrome. Obesity is considered as the initial trigger metabolic syndrome. Using the cut-off point 25 <BMI <30 defined overweight and a BMI> 30 obese, WHO reported that in 2014 more than 1.9 billion adults are overweight and 600 million of them obese or 39% overweight and 13% obese for adults. Obesity can occur in children, in 2013, 42 million children under five are overweight or obese. In Indonesia, pervalensi obesity and overweight also increased, from the data Riskesdas 2013 note that of the entire adult people of Indonesia 13.5% overweight and 15.4% obese, while for children 18.8% overweight and 8.8% obese. Morbidity of obesity can also be defined by using the parameters waist circumference, if the women waist circumference> 80 and men> 90 then defined as abdominal obesity. Riskesdas

ISBN 978-602-73538-0-0

2013 shows that abdominal obesity morbidity rate in Indonesia has increased as high as 18.8% in 2007 to 26.6% in 2013.

Insulin resistance if left untreated can develop into type 2 diabetes mellitus and cardio vascular disease. Insulin resistance cannot be cured, the most likely is treating obesity. Obesity management can be in two ways, namely non-pharmacologic by increasing physical activity and decreasing food intake, which is expected to be ideal body weight and pharmacologic by consuming drugs that can suppress hunger or improve insulin sensitivity. One of the drugs recommended by WHO for treating insulin resistance is metformin which has the function of lowering obesity lowers insulin resistance, hyperglycemia lowering, lowering blood pressure and decrease inflammation (Rojas and Gomes, 2013). Metformin has side effects occurrence of gastrointestinal disorders such as abdominal pain, flatulence and diarrhea (Anonymous, 1979). Other studies have shown the use of metformin for more than three years with a high dose (Wei Ting et al., 2006) cause 10-30% of patients treated with metformin decreased absorption of vitamin B12 (Callgahan et al., 1980), other data mentions decrease the absorption of vitamin B12 were 19% and 5% folic acid (Jager et al., 2010). Other side effects are sporadic consisting of vasculitis leukositoklatis and allergic pneumonitis (Klapholz et al., 1986), cholestatic jaundice (Desilets et al., 2001), and hemolytic anemia (Kashyap and Kashyap, 2000).

Side effects of the use of metformin, allowing it to look for herbal medicine as an alternative. Various studies of the extract of *Phyllanthus niruri* Linn. showed that the water extract of *P. niruri* are hypoglycemic, lowers cholesterol and triglycerides in rats induced by alloxan (Okoli et al., 2010) and streptozotocin-induced (Nwanjo, 2006), hypotensive in male rabbits (Amaechina and Omogbai, 2007), for the prevention and cure of disease or infection and degenerative (Oweyo et al., 2012), improve insulin resistance in rats induced a 10% sucrose (Adeneye, 2012), giving the effect of anti-apoptosis and inhibit inflammation and weight loss induced diabetic rats alloxan (Adeneye and Amole, 2006; Sheti et al., 2012).

Study the effects of *P. niruri* for the treatment of diabetes mellitus conducted in animals induced by streptozocin or alloxan. This is not in accordance with the development of insulin resistance or diabetes mellitus in humans, triggered by obesity. This study was conducted in Sprague Dawley rats obese. Rats made obese by giving additional intake in the form of liquid fructose and fat cattle on feed standard (AIN93G) and tap water *ad libitum*. Determination of obesity in rats using an index Lee obesity (Barnadis and Patterson, 1968). *P. niruri* extract dose used was 400 mg / kg body weight rats (Okoli, 2010; Giribabu, 2014) every morning for 14 days (Nwanjo, 2006; Asare and Addo, 2011).

ISBN 978-602-73538-0-0

EXPERIMENTAL

2.1 Animal

The current study was conducted on 30 healthy male sprague dawley rats weighing 158.73 ± 4,09 g. All animals were kept in standard conditions with constant 12h light/12h dark cycle at temperature of 25±2 °C. The rats were fed with standard food (AIN 93 G) and tap water *ad libitum.*

2.2 Plant Collection

Two kilograms of the fresh whole plants of *P. niruri* was collected from an abandoned arable land within Tasikmalya, within the month of October, 2014. The harvested plant materials were gently but thoroughly rinsed in tap water after which they were completely air-dried under shade and at room temperature (23–26 ^{0}C) in the laboratory for 2 weeks. The whole plants were pulverized into fine powder using the Laboratory Hammer-mill at the Department of Pharmacognosy, Faculty of Pharmacy, University of Muhammadiyah Puwokerto. The pulverized plant sample was then kept in air-tight and water-proof containers and kept in the refrigerator at 4 ^{0}C until needed for extraction.

2.3 Extraction Process

Two hundred grams of the pulverized sample material was completely extracted in 1 L of distilled water for 3 h using Soxhlet extractor obtained from the Experimental Laboratory at the Department of Pharmacognosy, Faculty of Pharmacy, University of Muhammadiyah Purwokerto. Soxhlet extraction of the pulverized plant material yielded a deep greenish-brown filtrate which was completely air-dried at 40 ^{0}C in a digital aerated oven leaving behind a deep brown, sweet-smelling solidr esidue. The process was repeated for four more timesto give a yield of 22.571.0%. The residues were all pooled into a dry, clean air-and water-tight containers and stored in the refrigeratorat 4 ^{0}C to prevent decomposition of the extract.

2.4 Animal Treatment

The first step (Induction of Obese) : 30 rats aged 6 weeks with an average body weight 158.73 ± 4.09 g g, randomly divided into 6 groups. The first group was fed a standard AIN-93-G and tap water ad libitum. Group II, III, IV, and V were fed standard AIN-93-G plus fructose and liquid beef tallow. Each week weight measured and calculated by the equation Lee index of obesity (Barnadis and Patterson, 1968):

$$Lee\ Index = \frac{\sqrt[3]{BB}}{PB} \times 1000$$

Otherwise obese rats when Lee index> 300 and obtained after 5 weeks.

The second step (treatment placement): after the rats in group II, III, IV, and V expressed obese, then determined for each treatment group.

ISBN 978-602-73538-0-0

Group I: normal rats were given distilled water 10 ml / kg body weight

Group II: obese rats were given distilled water 10 ml / kg body weight

Group III: obese rats were given water extract of *P. niruri* 400 mg / kg of body weight dissolved in distilled water

Group IV: Obese rats given metformin 45 mg / kg body weight dissolved in distilled water

Group IV: obese rats were given water extract of *P. niruri* 400 mg / kg body weight plus metformin 45 mg / kg body weight dissolved in distilled water

2.5 Measurement of Body Weight

Body weights of the treated rats were measured on the first, 8th and 15st day of the experiment with a mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 8th and 15st day inreference to the initial weight per group was calculated.

2.6 Biochemical Analyses

Amount of glucose, Trigllyserides and HDL in serum were determined using commercially available kits. Measurements were carried out in 14000 auto analyzer using manual colorimetric method.

2.7 Statistical Analysis

All values were expressed as mean± SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey tests and $p<0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

At the start of treatment, no significant differences in Lee index between groups indices Lee II, III, IV and V ($p>0.05$). The average body weight of rats in all groups experienced an increase when measured on the 8th and 15th day. The percentage increase in the average body weight of rats Group III, IV and V are not different from that of normal rats groups (Group I) ($p>0.05$) but is significantly different with obese rats groups (Group II) ($p=0.00$).

The average fasting blood glucose levels of obese rats before treatment> 164.48 ± 1.43 indicating that all of the rats obese were hyperglycemia because of insulin resistance. There is a significant difference in fasting blood glucose levels of normal rats (group I) with obese rats (Group II, III, IV, and V) ($p=0.000$). In the measurement of fasting blood glucose levels 8th day and the 15th day, there was a decrease in fasting blood glucose levels were significantly ($p=0.000$) in rats groups III, IV and V. In the normal group and obese rats were not treated there is an increase in fasting blood glucose levels but it was not significant ($p>0.05$) (Table 2 and Figure 1).

ISBN 978-602-73538-0-0

Tabel 1. Lee Index, body weight, % change body weight

Group	Lee Index	Body Weight (g)			% Change Body Weight	
		Day 1	Day 8	Day 15	Day 8	Day 15
I	286.10 ± 1.36	185.33 ± 2.98	192.17 ± 3.41	199.00 ± 3.68	3.68 ± 0.29	3.56 ± 0.49
II	310.25 ± 6.87	200.00 ± 5.28	210.33 ± 5.56	221.67 ± 5.58	5.17 ± 0.74	5.39 ± 0.35
III	317.34 ± 2.84	198.00 ± 2.86	205.33 ± 2.66	213.33 ± 2.80	3.71 ± 0.44	3.89 ± 0.43
IV	313.81 ± 8.71	201.83 ± 4.12	207.33 ± 4.10	212.83 ± 4.28	3.55 ± 0.25	3.35 ± 0.17
V	309.31 ± 3.71	199.83 ± 2.45	205.00 ± 2.68	210.50 ± 3.23	3.33 ± 0.31	3.15 ± 0.43

Group I : Normal rats + 10 ml/kg bw distilled water
Group II : Obese rats + 10 ml/kg bw distilled water
Group III : Obese rats + 400 mg/kg bw ekstrak P. niruri dissolved in distilled water
Group IV : Obese rats + 45 mg/kg bw metformin dissolved in distilled water
Group V : Obese rats + 400 mg/kg bw ekstrak P. niruri + 45 mg/Kg bw metformin dissolved in distilled water

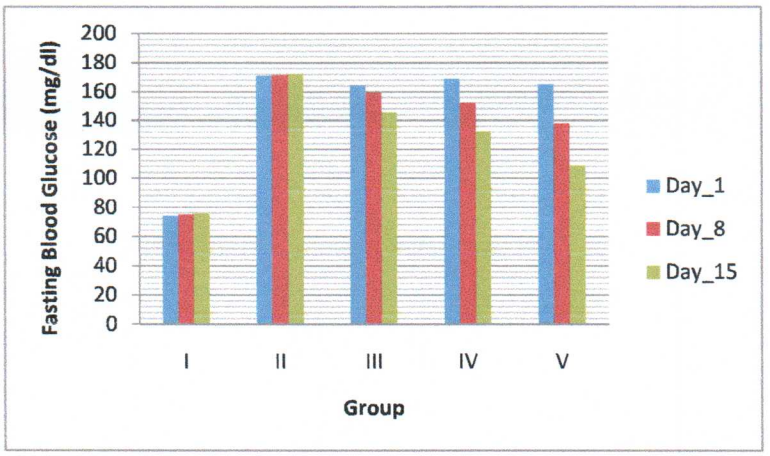

Fig 1. Fasting blood glucose level in normal and obese rats.

On the 15th, fasting blood glucose levels groups III, IV and V differ significantly with fasting blood glucose levels of normal rats (p=0.000) and obese rats group II (p=0.000). Hypoglycemic effect of extracts of *P. niruri* significantly different with effect of metformin (p=0.014) and effect of the combination of extracts of *P. niruri* with metformin (p=0.000), the hypoglycemic effect of metformin significantly different with the effects of combination of extracts of *P. niruri* with metformin (p=0.000).

Prior to treatment there is a significant difference in HDL levels in the group of normal rats with obese rats group (p=0.000). On the measurement the 8th and 15st visible HDL levels in groups III,

ISBN 978-602-73538-0-0

IV and V a significant increase ($p<0.05$), but in group I and II decreased levels of HDL were not significant (Table 2 and Figure 2).

Table 2. Fasting blood glucose, triglyserides and HDL

Group	Fasting Blood Glucose			Triglyserides			HDL		
	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15
I	74.33 ±0.96	74.83 ±1.05	75.43 ±9.58	81.14 ±1.36	81.53± 1.24	82.22 ±1.22	55.02 ±1.78	54.15 ±1.74	53.60± 1.62
II	171.04 ±4.74	171.59 ±4.67	172.25 ±4.67	132.48 ±1.09	133.56 ±1.26	134.51 ±0.99	25.21 ±1.42	24.90 ±1.54	24.60± 1.57
III	164.48 ±1.43	159.28 ±1.95	145.35 ±1.94	136.62 ±1.35	118.35 ±1.88	110.16 ±1.70	25.15 ±75.79	34.35 ±0.96	38.71± 1.08
IV	168.85 ±3.56	152.52 ±4.07	132.40 ±8.30	136.01 ±2.47	109.60 ±1.98	95.52 ±2.37	24.34 ±1.38	35.87 ±1.82	45.22± 2.01
V	165.04 ±4.37	137.87 ±6.19	108.60 ±6.09	136.01 ±2.14	110.27 ±3.09	91.59 ±2.79	26.07 ±0.91	36.42 ±1.72	50.54± 1.94

Group I : Normal rats + 10 ml/kg bw distilled water
Group II : Obese rats + 10 ml/kg bw distilled water
Group III : Obese rats + 400 mg/kg bw ekstrak P. niruri dissolved in distilled water
Group IIV : Obese rats + 45 mg/kg bw metformin dissolved in distilled water
Group IV : Obese rats + 400 mg/Kg bw ekstrak P. niruri + 45 mg/Kg bw metformin dissolved in distilled water

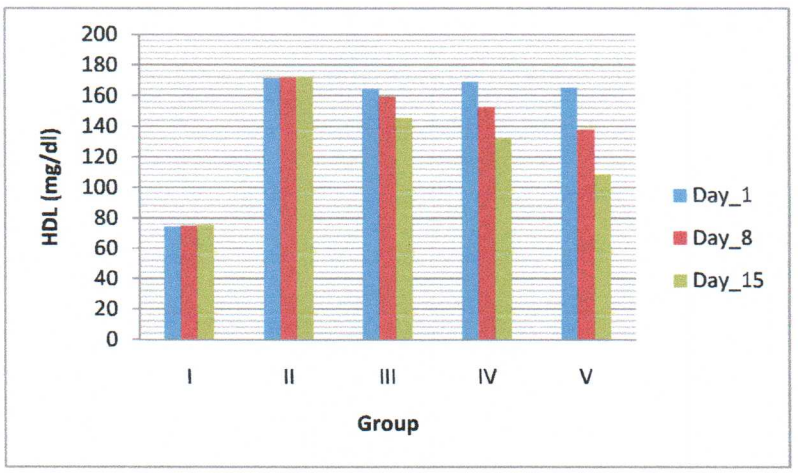

Fig 2. HDL level in normal and obese rats.

On 15th day, the levels of HDL rats given the extract of *P. niruri*, and metormin significantly different to normal rats group ($p=0.000$), but rats fed a combination of *P. niruri* and metformin did not differ ($p=0.136$). but HDL levels of all groups of rats treated with HDL levels significantly different from obese rats that were not given any treatment ($p=0.000$). Extracts of *P. niruri*,

ISBN 978-602-73538-0-0

metformin and combination extracts of *P. niruri* and metformin give different effects significantly elevated levels of HDL ($p<0.05$).

Before treatment, significantly difference of triglyceride levels in the group of normal rats with rats obese group ($p=0.000$). After 14 days of treatment in the groups III, IV and V decreased TG levels were significantly ($p<0.05$), but in groups I and II. there are elevated levels of triglycerides were not significant (Table 2 and Fig 3).

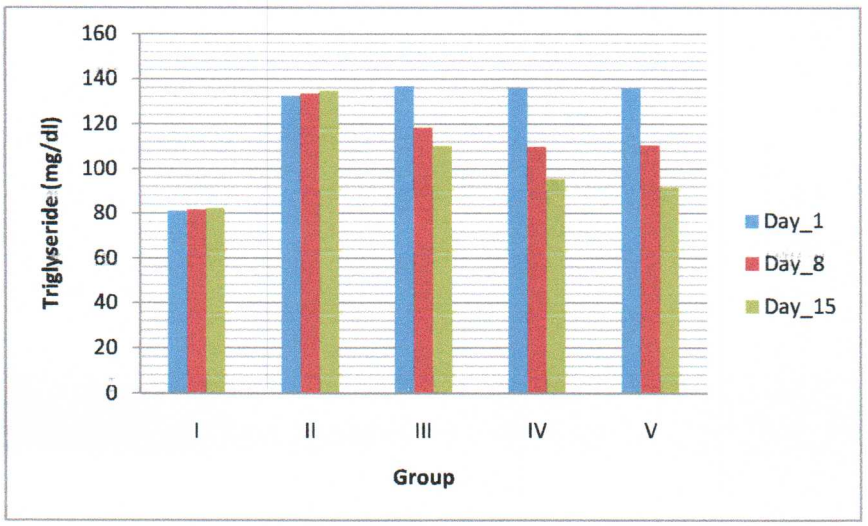

Figure 3. Triglyserides level in normal and obese rats.

On the 15th day, occurred a significant decrease in triglyceride levels in rats treated, extracts of *P. niruri*. Metformin and a combination of both ($p = 0.000$), although still significant different to normal rats group ($p=0.000$), as well as groups of obese rats who were not given any treatment ($p=0.000$). Effects of extract of *P. niruri* significantly different with metformin and combination effects *P. niruri* with metformin ($p=0.000$), as well as the effects of metformin were significantly different to the effect of the combination of *P. niruri* and metformin ($p=0.046$).

WHO recommends metformin as drug type 2 diabetes mellitus, because metformin can reduce obesity, lowers insulin resistance, hyperglycemia lowering, lowering blood pressure and decrease inflammation (Rojas and Gomes, 2013). Various studies of the extract of *P. niruri* showed that the water extract of *P. niruri* are possess hypoglycemic effect, lowers cholesterol and triglycerides in rats induced by alloxan (Okoli et al., 2010) and streptozotocin-induced (Nwanjo, 2006), hypotensive in male rabbits (Amaechina and Omogbai, 2007), for the prevention and cure of disease or infection and degenerative (Oweyo et al., 2012), improve insulin resistance in rats induced a 10% sucrose (Adeneye, 2012), giving the effect of anti-apoptosis and inhibit

ISBN 978-602-73538-0-0

inflammation and weight loss induced diabetic rats alloxan (Adeneye and Amole, 2006; Sheti et al., 2012).

CONCLUSIONS

Water extract of *P. niruri*, metformin, and combination of both give the effect of controlling weight gain that did not differ from that of normal rat groups ($p>0.05$), lower blood glucose levels, increase HDL and lowering triglycerides with different effects ($p<0.05$) and the largest effect is given by the combination of the water extract of *P. niruri* and metformin. The extract of *P. niruri* effectively used with metformin to improve insulin resistance in obesity.

ACKNOWLEDGMENT

The author wishes to acknowledge the support of knowledge of Prof. Edi Dharmana, Prof. Soeharyo, Prof. Ign Riwanto, and Dr. Tjokorda lecturers of the Faculty of Medicine, University of Diponegoro, technical support of Mr. Yulianto as head of the laboratory of Nutrition PAU UGM.

REFERENCES

Abel E.D. (2010) Free fatty acid oxidation in insulin resistance and obesity. *Heart Metab*. 48:5–10.

Adeneye A.A., Amole O.O. et al. (2006) Hypoglycemic and hypocholesterolemi cactivities of the aqueous leaf and seed of *Phyllanthus amarus* in rats. *Fitoterapia* 77:511–514.

Adeneye A.A. (2012) The leaf and seed aqueous extract of *Phyllanthus amarus* improves insulin resistence diabetes in experimental animal sudies. *Journal of Ethnopharmacology* 144: 705-711.

Amaechina F.C., Omogbai E.K. (2007) Hypotensive effect of aqueous extract of the leaves of *Phyllanthus amarus* Schum and Thonn (Euphorbiaceae). *Acta Poloniae Pharmaceutica ñ Drug Research* 64(6):547-552.

Anonymous (1979) Metformin: a review of its pharmacological properties and therapeutic use. *Diabetes Metab*. 5:233–245.

Asare G.A., Addo P., et al. (2011) Acute toxicity studies of aqueous leaf extract of *Phyllanthus niruri*. *Interdiscip. Toxicol.* 4(4):206-210.

Bernardis L.L., Patterson B.D. (1968) Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. *J Endocrinol*. 40:527-528.

Callaghan T.S., Hadden D.R., Tomkin G.H. (1980) Megaloblastic anemia due to vitamin B12 malaborsption associated with long-term metformin treatment. *Br Med J*. 280:1214–1215.

Desilets D.J., Shorr A.F., Moran K.A., Holtzmuller K.C. (2001) Cholestatic jaundice associated with the use of metformin. *Am J Gastroenterol.* 96:2257–2258.

ISBN 978-602-73538-0-0

Giribabu N., et al. (2014) Aqueous extract of *Phyllanthus niruri* leaves displays in vitro antioxidant activity and prevent the elevation of oxidative stress in the kidney of streptozotocin - induced diabetic male rats. *Hindawi Publishing Corporation*. 3.

Jager J., Kooy A., Lehert P., Wulffelé M. et al. (2010) Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo controlled trial. *BMJ*. 340:c2181.

Kashyap A.S., Kashyap S. (2000) Haemolytic anaemia due to metformin. *Postgrad Med J.* 76:125–126.

Klapholz L., Leitersdorf E., Weinrauch L. (1986) Leucocytoclastic vasculitis and pneumonitis induced by metformin. *BMJ.* 293:483.

Nwanjo H.U. (2006) Studies on the effect of aqueous ectract of *Phyllanthus niruri* leaf on plasma glucose level and some hepatospecific markers in diabetic wistar rats. *The Internet Journal of Laboratory Medicine* 2(2).

Okoli, C.O., Ibiam A.F. et al. (2010) Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. *African Journal of Biotechnology* 9(2):248-259.

Oweyo et al. (2012) Immunomodulation capabilities of aqueous leaf extract of *Phyllanthus amarus* in male wistar rats. *Report and Opinion*. 4(1): http://www.sciencepub.net/report.

Rojas and Gomes (2013) Metformin: An old but still the best treatment for type 2 diabetes. *Diabetology & Metabolic Syndrome* 5(6):1-15.

Sheti et al. (2012) Antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic rats. *Asian Journal of Plant Science and Research*. 2(1):11-15

Wei Ting R.Z., Szeto C.C., Chan M., Ma K. et al. (2006) Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Intern M.*