International Journal of Innovation, Creativity and Change. www.ijicc.net
Volume 9, Issue 1, 2019

Effective Combination of Phyllanthus Niruri Linn. and Metformin to Improve Insulin Resistance in Obese Rats

A S Kosnayani[a*], AK Hidayat[b], Darmana E[c], I Riwanto[d], S Hadisaputro[e], [a,b]Siliwangi University, Tasikmalaya, East Java, Indonesia, [c,d,e]Diponegoro University, Centre Java, Indonesia, Email: [a*]aisrikosnayani@unsil.ac.id

Obesity is always relevant to insulin resistance. Insulin resistance, if not treated, will develop into Diabetes Mellitus (DM) or Cardiovascular Disease (CVD). Insulin resistance cannot be cured but controlled, through the management of obesity, with non-pharmacological or pharmacological approaches, such as using metformin. Metformin can reduce weight, blood glucose, and improve the lipid profile. However, it still has the disadvantage of indigestion and decreases the absorption of vitamin B12. Therefore, it is necessary to find an alternative form of herbal medicine. Phyllanthus niruri Linn. is known for being a hypoglycaemic, hypotensive, antioxidant that can control weight. This study aims to determine the effect of combination extracts of P. niruri, and metformin to improve insulin resistance in obese rats. The effect of repeated administration of a combination water extract of P. niruri 400 mg/kg BW and metformin 45 mg/kg BW was evaluated in obese Sprague Dawley rats and the placebo group. The indicators measured were Lee Obesity Index, fasting blood glucose, insulin, and insulin resistance. They were measured pre, after seven days, 14 days, 21 days and 28 days. A combination water extract of P. niruri, can give the effect of a lower Lee Obesity Index and differ from the control group (placebo) ($p>0.05$), lower blood glucose levels, insulin and insulin resistance (Homa_IR) with different effects ($p<0.05$). It was concluded that the combination water extract of P. niruri and metformin was effective to improve insulin resistance in obese rats.

Key words: *Insulin Resistance, Obesity, Diabetes*

Introduction

Obesity is defined as a nutritional disorder that is characterised by the excess or abnormal accumulation of fat that could affect health. Obesity is associated with insulin resistance (Abel, 2010). Obesity and insulin resistance are always accompanied by hypertension, hypertriglyceridemia, and low HDL; a condition called metabolic syndrome (Reaven, 1988). Obesity is considered as the initial trigger of 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 per cent of adults are overweight and 13 per cent are obese (WHO, 2015). Obesity can also occur in children. In 2013, 42 million children under five were overweight or obese. In Indonesia, the prevalence of being obese or overweight also increased. The Riskesdas 2013 data notes that of the entire adult population of Indonesia, 13.5 per cent were overweight and 15.4 per cent were obese. While for children, 18.8 per cent were overweight and 8.8 per cent were obese (Kemenkes, 2014). The morbidity of obesity can also be defined by using the parameters waist circumference. If women's waist circumference is > 80 and men's is > 90, then it is defined as abdominal obesity. The Riskesdas 2013 data also shows that the abdominal obesity morbidity rate in Indonesia has increased from as high as 18.8 per cent in 2007 to 26.6 per cent in 2013.

Insulin resistance, if left untreated, can develop into Type 2 Diabetes Mellitus and Cardiovascular Disease. Insulin resistance cannot be cured; the most likely option is to treat obesity. Obesity management can be achieved in two ways. Firstly, non-pharmacological approaches by increasing physical activity and decreasing food intake, which is expected to achieve an ideal body weight. Secondly, pharmacological approaches 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, insulin resistance, hyperglycemia, and blood pressure, as well as decreases inflammation (Rojas & Gomes, 2013). Metformin has known side effects of gastrointestinal disorders such as abdominal pain, flatulence and diarrhea (Hermann, 1979). Other studies have shown the use of metformin for more than three years on a high dose (Wei Ting, 2006) can cause in 10–30 per cent of patients, decreased absorption of vitamin B12. (Callaghan, 1980) Other data also mentions the decreased absorption of vitamin B12 at 19 per cent and folic acid at 5 per cent (Jager, 2010). Other side effects are sporadic, consisting of leukocytoclastic vasculitis and allergic pneumonitis (Klapholz, 1986), cholestatic jaundice (Desilets, 2001), and hemolytic anemia (Kashyap & Kashyap, 2000).

Due to the side effects experienced from the use of metformin, it enables looking to herbal medicine for an alternative. Various studies of the extract of Phyllanthus niruri Linn. showed that the water extract of P. niruri is hypoglycemic, lowers cholesterol and triglycerides in rats

induced by alloxan (Okoli, 2010) and streptozotocin (Nwanjo, 2006); it is hypotensive in male rabbits (Amaechina and Omogbai, 2007) for the prevention and cure of degenerative disease or infection (Oweyo, 2012); improves insulin resistance in rats induced with a 10 per cent sucrose (Adeneye, 2012), giving the effect of anti-apoptosis; and inhibits inflammation and weight loss in induced diabetic rats on alloxan (Adeneye, 2006; Sheti, 2012).

This study explores 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 obese Sprague Dawley rats. The rats were made obese by providing them with additional intake in the form of liquid fructose and fat cattle on feed standard (AIN93G) and tap water ad libitum. The determination of obesity in rats was made using the Lee Obesity Index (Bernardis and Patterson, 1968). A P. niruri extract dose of 400 mg/kg body weight in rats (Okoli, 2010; Giribabu, 2014) was used every morning for 14 days (Nwanjo, 2006; Asare, 2011). Therefore, the purpose of this study is to determine the effect of combination extracts of P. niruri, and metformin to improve insulin resistance in obese rats.

Methods

This research used a multigroup time series design. The time-series design was based on the application of multiple measurements, before and after treatment, to document patterns or trends in behaviour or treatment outcomes by using experimental animals as research subjects.

Animal

The current study was conducted on 30 healthy male *sprague dawley* rats weighing 158.73 ± 4.09g. All animals were kept in standard conditions with the constant 12h light/12h dark cycle at a temperature of 25±2°C. The rats were fed with standard food (AIN 93 G) and tap water ad libitum.

Plant collection

Two kilograms of fresh whole plants of P. niruri were collected from abandoned arable land within Tasikmalaya, 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 (23–26ºC) in the laboratory for two weeks. The whole plants were pulverised into a fine powder using a Laboratory Hammer-mill at the Department of Pharmacognosy, Faculty of Pharmacy, and University of Muhammadiyah Puwokerto. The pulverised plant sample was then kept in air-tight and water-proof containers in the refrigerator at 4ºC until needed for extraction.

Extraction process

Two hundred grams of the pulverised sample material was completely extracted in 1L of distilled water for 3h using a Soxhlet extractor obtained from the Experimental Laboratory at the Department of Pharmacognosy, Faculty of Pharmacy, University of Muhammadiyah Purwokerto. The Soxhlet extraction of the pulverised plant material yielded a deep greenish-brown filtrate which was completely air-dried at 40ºC in a digital aerated oven, leaving behind a deep brown, sweet-smelling solid residue. The process was repeated four more times to provide a yield of 22.571.0 per cent. The residues were all pooled into dry, clean, air and water tight containers and stored in the refrigerator at 4ºC to prevent the decomposition of the extract.

Animal treatment

The first step (Induction of Obesity): 30 rats aged six weeks with an average body weight of 158.73 ± 4.09g, were randomly divided into six groups. The first group was fed a standard AIN-93G and tap water ad libitum. Group two, three, four, and five were fed standard AIN-93-G plus fructose and liquid beef tallow. Each week the rats were weight measured and calculated against the Lee Index of Obesity equation (Bernardis and Patterson, 1968):

$$Indeks\ Lee = \frac{\sqrt[3]{BB}}{PB} \times 1000 \qquad (1)$$

Otherwise, obese rats when Lee index > 300 and obtained after five weeks. The second step (treatment placement).

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

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

Measurement of Lee index

The length and body weights of the treated rats were measured on the first, 8th, 15th, 21st and 28th day of the experiment with a ruler and a Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 8th and 15th day, in reference to the initial weight per group, was calculated.

1.1. Biochemical analysis

The amount of glucose was determined with the enzymatic method, and insulin in serum was determined using commercially available kits (ELISA). Measurements were carried out in an 14000 auto analyser using the manual colorimetric method.

Measurement insulin resistance

$$Homa\_IR = \frac{Fasting\ glucose\ \left(\frac{mg}{dL}\right) x\ insulin\ (\mu\frac{U}{mL})}{405} \qquad (2)$$

Statistical analysis

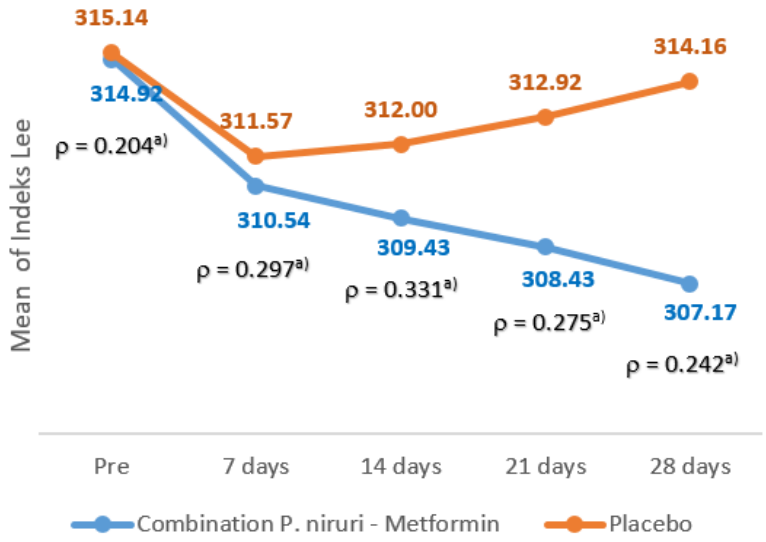
All values were expressed as mean ± SEM. The differences were compared using one-way analysis of variance (ANOVA), followed by Tukey tests and the Mann-Whitney test, followed by the Kruskal-Wallis test ($p<0.05$ were considered statistically significant).

Results

Lee Index

At the pre-treatment stage, there were no significant differences in the Lee Index between groups. The mean of the Lee Index at the pre-stage, after 7, 14, 21, and 28 days of treatment can be seen in Figure 1.

Figure 1. Mean of the Lee Index at any time of measurement; [a)] Mann-Whitney test followed by Kruskal-Wallis test

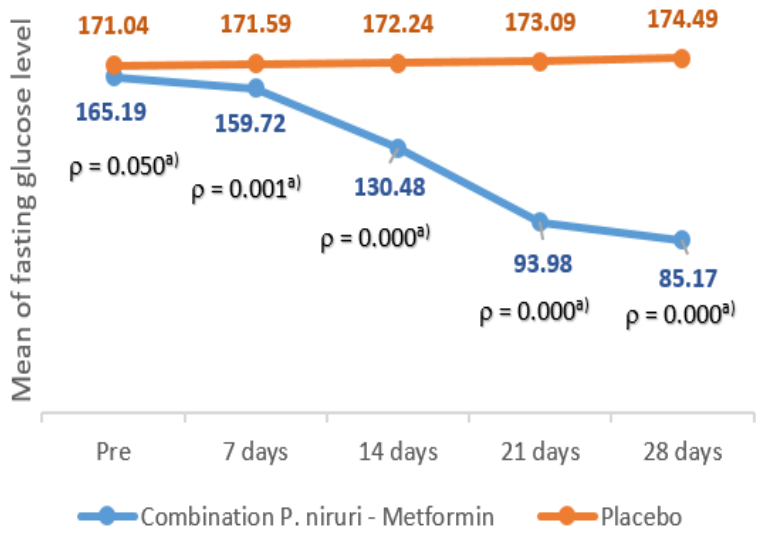


The mean Lee Index of rats in the experiment group decreased when measured after 7, 14, 21, and 28 days of treatment, however, the control group saw an increase. There was no difference between the Lee Index of the experimental and the control group at each measurement time (ρ>0.05).

Fasting glucose level

At the pre-treatment stage there were no significant differences in the fasting glucose level between groups. The mean fasting glucose level at the pre-stage, after 7, 14, 21, and 28 days of treatment can be seen in Figure 2.

Figure 2. Mean of fasting glucose level at any time of measurement; [a)] Mann-Whitney test followed by Kruskal-Wallis test.

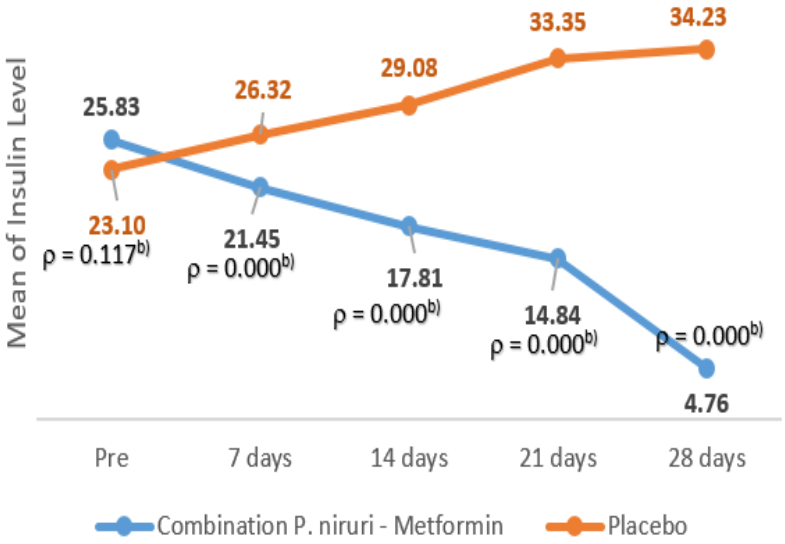


After 7, 14, 21 and 28 days of treatment, the fasting glucose content of rats administered with a combination of *P.niruri* and metformin decreased, while the control group increased. There was a difference of mean fasting glucose level between groups of rats given a combination of *P. niruri* water extract and metformin within the control group (ρ<0.05)

Insulin level

At the pre-treatment stage there were no significant differences in the insulin level between groups. The mean insulin level at the pre-stage, after 7, 14, 21, and 28 days of treatment can be seen in Figure 3.

Figure 3. Mean of insulin level at any time of measurement; [b)] ANOVA Post Hoc followed by Tukey test

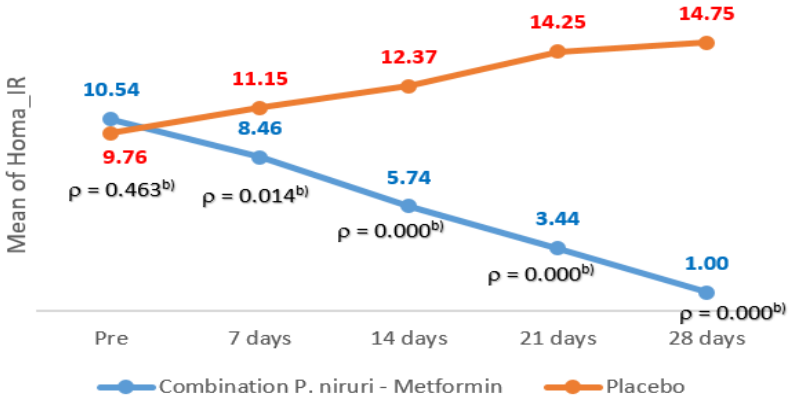


After 7, 14, 21 and 28 days of treatment, the insulin level content of rats administered with a combination of *P.niruri* and metformin decreased, while the control group increased. There was a difference of mean insulin between groups of rats given a combination of *P. niruri* water extract and metformin within the control group ($\rho<0.05$)

Insulin resistance (Homa_IR)

At the pre-treatment stage there were no significant differences in Homa_IR between groups. The mean Homa_IR at the pre-stage, after 7, 14, 21, and 28 days of treatment can be seen in Figure 4.

Figure 4. Mean of Homa_IR at any time of measurement; [b)] ANOVA Post Hoc followed by Tukey test.



After 7, 14, 21 and 28 days of treatment, the Homa_IR content of rats administered with a combination of *P.niruri* and metformin decreased, while the control group increased. There was a difference of Homa_IR between groups of rats given a combination of *P. niruri* water extract and metformin within the control group ($\rho<0.05$).

Discussion

Administering a combination of water extract meniran 400mg/kgBB and metformin 45mg/kg BW can reduce the Lee Index, although, not to a significantly different degree with the group of obese mice who were given a placebo. The Lee's Index decline showed control over the weight gain in obese rats.

Giving a combination of water extract meniran 400mg/kgBB and metformin 45mg/kg body weight can reduce fasting glucose and insulin. A decrease in fasting glucose and insulin will eventually lower Homa_IR. The decrease in Homa_IR showed an improvement in the condition of insulin resistance of obese rats.

WHO recommends metformin as a drug for the treatment of Type 2 Diabetes Mellitus, because metformin can reduce obesity, lowers insulin resistance, hyperglycemia, and blood pressure, and can also decrease inflammation (Rojas and Gomes, 2013). Various studies of the extract of P. niruri showed that the water extract of P. niruri possesses a hypoglycemic effect, and lowers cholesterol and triglycerides in rats induced by alloxan (Okoli, 2010) and streptozotocin (Nwanjo, 2006); it is hypotensive in male rabbits (Amaechina and Omogbai, 2007), for the prevention and cure of degenerative disease or infection (Oweyo, 2012); improves insulin resistance in rats induced with a 10 per cent sucrose (Adeneye, 2012), giving the effect of anti-apoptosis; and inhibits inflammation and weight loss induced diabetic rats on alloxan (Adeneye, 2006; Sheti, 2012).

Conclusion

The combination of water extract of *P. niruri* and metformin provided the effect of controlling weight gain that did not differ from the experiment and the control group ($p>0.05$), lowered blood glucose levels, and lowered insulin with different effects ($p<0.05$). The extract of *P. niruri* was effectively used with metformin to improve insulin resistance in obesity.

REFERENCES

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

Adeneye, A. A. (2006). Hypoglycemic and hypocholesterolemi cactivities of the aqueous leaf and seed of Phyllanthus amarus in rats. *Fitoterapia,* **77:** 511–14.

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:** 705–11.

Amaechina, F. C. and 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): 547–52.

Asare, G. A. (2011). Acute toxicity studies of aqueous leaf extract of Phyllanthus niruri. *Interdiscip Toxicol,* **4**(4): 206–10.

Bernardis, L. L. and Patterson, B. D. (1968). Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. *J Endocrinol,* **40:** 527–28.

Callaghan, T. S. (1980). Megaloblastic anemia due to vitamin B12 malaborsption associated with long-term metformin treatment, *Br Med J.* **280** 1214–15.

Desilets, D. J. (2001). Cholestatic jaundice associated with the use of metformin. *Am J Gastroenterol,* **96** 2257–58.

Giribabu, N. (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. *Evidence-Based Complementary and Alternative Medicine* **2014** 1–10.

Hermann, L. S. (1979). Metformin: A review of its pharmacological properties and therapeutic use *Diabetes Metab*. **5** 233–45.

Jager, J. (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. and Kashyap, S. (2000). Haemolytic anaemia due to metformin. *Postgrad Med J.* **76:** 125–6.

Kemenkes, R.I. (2014). *2013 National Basic Health Research (RISKESDAS) report (Jakarta: Health Research and Development Agency).*

Klapholz, 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): 1–6.

Okoli, C. O. (2010). Evaluation of antidiabetic potentials of Phyllanthus niruri in alloxan diabetic rats. *African Journal of Biotechnology,* **9**(2): 248–59.

Oweyo, (2012). Immunomodulation capabilities of aqueous leaf extract of Phyllanthus amarus in male wistar rats. *Report and Opinion,* **4**(1): 22–37.

Reaven, (1988). *Insulin Resistance The Syndrome X Contemporary Endrocynology* (New York: Humana Press).

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

Sheti, (2012). Antidiabetic effect of ethanolic leaf extract of Phyllanthus amarus in alloxan induced diabetic rats. *Asian Journal of Plant Science and Research,* **2**(1): 11–5.

Wei Ting, R.Z. (2006). Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Intern M.* **166** 1975–9.

WHO, (2015). *Global Health Observatory (GHO) data Obesity* (Geneva).

Acknowledgment

The author wishes to acknowledge the support of knowledge of Prof. Dr. Hardhono Soesanto, Sp.PA(K), *Prof. dr. Lisyani* Suromo, *Sp.PK* (K), Dr. dr. Tjokorda Gde Dalem Pemayun, Sp.PD-KEMD of the Faculty of Medicine, University of Diponegoro, Prof. Dr. dr. JB. Soeparyatmo, Sp.PK(K), of the Faculty of Medicine, UNS, technical support of Mr. Yulianto as head of the laboratory of Nutrition PAU UGM.