

A Doubled-Blind, Crossover-RCT in T2DM for Evaluating Hypoglycemic effect of P. indicus, M. charantia, P. vulgaris and A. paniculata in Central Java

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A Doubled-Blind, Crossover-RCT in T2DM for Evaluating Hypoglycemic effect of *P. indicus*, *M. charantia*, *P. vulgaris* and *A. paniculata* in Central Java

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Abstract

This study investigated whether mixture extract of *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata* could lower plasma glucose in Type 2 Diabetes Mellitus (T2DM) patients. 43 T2DM patients, consisting of 10 male and 33 female patients participated in this positive-controlled, double-blind, and crossover clinical study with administration of either mixture extract (22 mg/kg BW) or glibenclamide 5 mg daily at breakfast time. Treatment of extract or glibenclamide was administered for a month, then medication was changed after a week of washout period and finally combination therapy was administered after wash out period as well. The efficacy of mixture extract was measured by using Fasting Plasma Glucose (FPG) and two-hour postprandial Plasma Glucose (PPG). Forty-one subjects completed the study. Data was analyzed using SPSS 19 with Student's *t* test, and $p < 0.05$ was considered significant. FPG level significantly decreased 16.07 mg/dl after extract, 43.34 mg/dl after glibenclamide and 50.16 mg/dl after combination treatment. Two-hour PPG level decreased 25.88 mg/dl after extract, 66.61 after glibenclamide and 58.93 after combination treatment. We concluded that extract administration could lower FPG and PPG although not as good as glibenclamide treatment while combination treatment was the best to lower FPG.

Keywords: Anti-Diabetic, Clinical-Trial, Extract, Herbal-Medicine

1. Introduction

The global prevalence of diabetes among adults has risen from 4.7% in 1980 to 8.5% in 2014¹. Prevalence of T2DM

in Indonesia is 7.5% in 2014, increased from 1.5-2.3% in 1980². Complementary and alternative medicine is involved in management of T2DM by using herbs and supplements as alternative, besides the common use of

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western medical treatment. Studies estimated that 30% of T2DM patients use complementary and alternative medicine³. Medicinal plants are important part of therapeutic aid for alleviating the ailments of human kinds. Herbs have been used to treat diabetes since a long time. WHO has listed more than 20,000 plants around the world which are commonly used and potentially believed to have anti diabetic effects⁴.

Pterocarpus indicus (Fabaceae), *Momordica charantia* (Cucurbitaceae), *Phaseolus vulgaris* (Fabaceae) and *Andrographis paniculata* (Acanthaceae) are Asian authentic herbs which have anti diabetic effects. Some studies report the use of extract *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata* both as a mixture or single extract respectively to lower plasma glucose in diabetic mice model^{3,5}. In pre clinical trial, *Andrographis paniculata* Nees extract has the most effect for lower blood glucose in diabetic mice model⁶. The use of *Momordica charantia* extract was also reported to lower blood glucose in diabetic rat model as well as glibenclamide treatment positive control^{7,8}. Our previous study reported that mixture extract of *Pterocarpus indicus* 20%, *Momordica charantia* 10%, *Phaseolus vulgaris* 40% and *Andrographis paniculata* 30% showed significantly hypoglycemic effect on diabetic mice model⁹. Our study also concluded that lethal dose 50% of this mixture extract was 20 g/kgBW. Effective dose to reduce blood glucose was 27 mg/200 gBW equivalent to the dose of glibenclamide 0.13 mg/200 gBW or 5 mg for average dose in human. Chronic toxicity test conducted using dose up to 504 mg/200 gBW once daily for 4 months had no alteration in liver and renal function test nor hematologic parameters. This study also concluded that potential mechanism of these herbs was in sensitizing β cell pancreas to release insulin, because its effect to lower blood was proportional with effect of glibenclamide⁹.

Despite several studies of antidiabetic agent in animal model study, there are limited clinical trial studies to validate its effects in humans. Therefore, investigating the effect of mixture extract of *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata* to lowering plasma glucose can be one of alternative treatment in T2DM patients.

2. Material and Methods

2.1 Extract Preparation

Ethanol extraction from *Pterocarpus indicus* folium 20%, *Momordica charantia* fructus 10%, *Phaseolus vulgaris* fructus 40% and *Andrographis paniculata* Nees 30% prepared and packaged by PT Njonja Meneer as *Diabmeneer*[®].

2.2 Study Design

The study was designed as a prospective, randomized, doubled-blind, comparative positive controlled, cross-over, clinical trial. Treatment group was administered with a dose of mixture extract *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata*. Positive control group was administered a daily dose of glibenclamide (5 mg). After cross-over switching trial. Both groups received combination treatment of mixture extract and glibenclamide.

2.3 Ethic and Regulatory Approvals

Prior to study initiation, the study protocol was approved by the Medical Research Ethics Committee at Faculty of Medicine Diponegoro University and Dr. Kariadi Hospital Semarang (Protocol number 87, September 2009). Trial also was registered at University Hospital Medical Information Network (UMIN) Clinical Trial Registry <http://www.umin.ac.jp> (UMIN000022442).

2.4 Study Population (Total Patients)

Forty-three T2DM men and women patients in Internal Medicine Clinic, Sultan Agung Islamic Hospital, Semarang met the inclusion criteria inclusion criteria was determined as: new or recently diagnosed T2DM men and women patients, 30–60 years, who underwent routine treatment. Random Plasma Glucose (RPG) should be less than 400mg/dl. Subjects must have no insulin therapy. If someone has undergone treatment program to control his/her blood glucose, treatment should be stopped temporarily for a week as washing out period continues and medical examination by a doctor determines eligibility to participate in this study.

HbA1C level should be between 7-12mg/dl, should have no severe complication and any other condition that would disturb the study, i.e.: pregnancy, hypoglycemic coma, severe infection and abnormal incomplete blood analysis, abnormal liver function test (SGPT), abnormal kidney function test (creatinin), abnormal ECG, received other medication other than those approved by internal medicine team to stop medication temporarily based on medical indication. Subjects who developed complications or harmful conditions as well as subjects who did not heed to the instructions were excluded from the study.

2.5 Intervention

Screening was performed to acquire eligible subjects to participate in the study. Subjects received instruction about the study and then signed the *informed consent*. Instruction includes to stop all medication for a week before treatment. Diet program was adjusted with daily routine activity by clinical nutritionist who was involved in the research and were self recorded daily. Diet and physical activities were monitored twice a week over phone and once a week directly by investigator to ensure calory intake and output met the program. Subjects were randomly assigned to group A and Group B. Group A received treatment by a daily 22mg/kg BW doses of mixture extract. Positive control group was administered a daily glibenclamide 5mg¹⁰ (*Indofarma*[®]). Both treatments were administered with the same preparation which was coded and known only to the researcher. They were taken during meal at breakfast time for a month. Treatment continued to wash out period for a week, then subjects were changed to receive switch treatment also for a month. After a week of second washing period, subjects received combination preparation of both mixture extracts and glibenclamide 5mg for a month as well.

2.6 Randomization

A random allocation list was generated. According to randomization list, 50% T2DM patients were allocated to group A that would receive extract treatment first and the rest of T2DM patients were allocated to group B that would receive glibenclamide administration. For each T2DM patient, the doctor involved in the study at

the outpatient clinic received a sealed preparation to be given to a randomly chosen patient, as mentioned earlier.

2.7 Outcome Parameters

The objective of present study was to assess the efficacy of hypoglycemic effect extract in T2DM patients. Parameters measured for each phase before and after treatment were Fasting Plasma Glucose (FPG) and 2 hours Postprandial Plasma Glucose (PPG). Blood analyses for 2 hours PPG was drawn after consuming a standard-meal as programed by clinical nutritionist. PG and other biochemical parameters were measured by Cobas Integra (Roche).

2.8 Sample Size

A minimal sample size of 20 T2DM patients in each group provided a power of 90% assuming a significance level of 0.05.

2.9 Statistical Analyses

Results were expressed as mean \pm Standard Deviation (SD). The difference in FPG and PPG before and after treatment was assessed using Student's *t*-test. Significance was considered at $p \leq 0.05$. All statistical analyses were performed by blinded stastician to avoid conflict of interest, using SPSS version 19 (IBM SPSS Inc. Chicago, USA).

3. Results

3.1 Patient Population

Out of a total of 43 eligible T2DM patients, 21 were allocated to Group A and 22 were allocated to Group B. (Figure 1). One patient each from both the the Groups A and B, did not come back for the next visit, and was terminated from the study. Forty-one T2DM patients met the program compliance and completed the study. Therefore, analysis includes forty-one subjects. Table 1 summarizes clinical characteristics of T2DM patients in the study. Overall there were no relevant statistical differences between both groups at baseline (all $p > 0.05$). Standard Deviation(SD) for most clinical characteristics are shown in wide range as the recruited subjects were limited and hence more homogenous characteristics

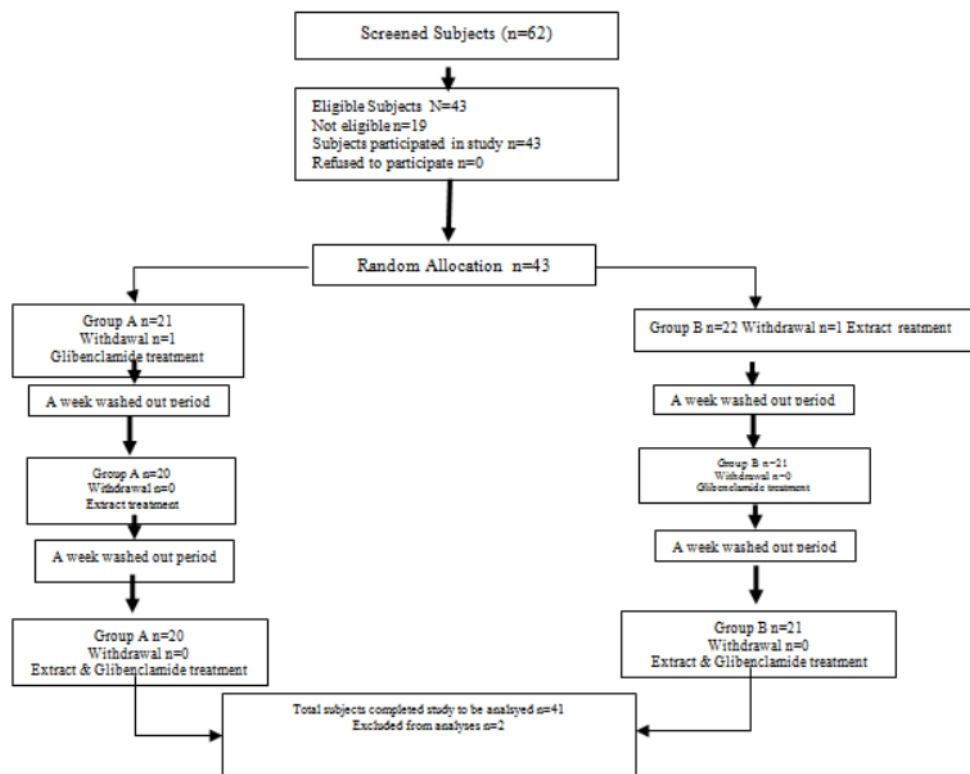


Fig. 1. CONSORT diagram of T2DM patients in the study.

Table 1: Clinical characteristics between the groups participating in the study^{a,b}

	Group A	Group B	pt	Total
	n=21	n=20		n=41
Age (years)	49.81±7.41	49.75±6.97	0.979	49.78±7.11
Sex			0.466††	
Male (%)	4(19)	5(25)		9(22)
Female (%)	17(81)	15(75)		32(78)
Systolic BP (mmHg)	146.19±24.29	132.00±27.83	0.090	139.27±26.78
Diastolic BP (mmHg)	85.71±12.07	83.50±21.83	0.668	84.63±17.34
Heart Rate (times/minute)	87.52±7.32	84.70±9.60	0.295	86.15±8.53
BMI (kg/m ²)	26.44±10.45	26.59±4.48	0.951	26.51±8.00
Respiratory Rate (times/minute)	20.57±1.8	21.2±1.88	0.281	20.88±1.85
Random Plasma Glucose (mg/dl)	175.48±12.04	170.30±56.89	0.769	172.79±55.38
HbA1C	9.16±1.73	8.67±1.63	0.289	8.91±1.67
SGPT	18.76±8.23	19.66±10.45	0.754	19.17±9.30
Creatinin	0.75±0.40	1.09±0.39	0.405	0.91±0.38
FPG (mg/dl)	198.22±93.35	184.90±83.59	0.453	196.68±82.67
PPG (mg/dl)	241.24±113.98	244.05±103.04	0.610	254.10±102.92

^adata are means±SD or proportions. ^bBMI, body mass index; HbA1C, hemoglobin A1C; SGPT, serum glutamic-pyruvic transaminase; FPG, fasting plasma glucose; PPG, postprandial plasma glucose. †statistical differences by t test or ††Chi-Square between group A and group B

could not be determined. There were **no adverse events during the study** as per our clinical monitoring of the subjects for physical examination, weight loss, liver and renal function parameters and hypoglycemic condition.

3.2 Changes in Plasma Glucose

FPG in all analysed treatment group were significantly decreased (Table 2). Mean FPG in Glibenclamide group decreased from 199.54±94.62 mg/dl to 156.20±66.90 mg/dl, p:0.001, Extract group decreased from 197.27±89.70 to 181.20±76.02, p:0.037 and Combination treatment decreased from 204.73±93.96 mg/dl to 208.32±89.45 mg/dl, p:0.001 (Figure 2).

Table 2: The changes in FPG and PPG before and after treatment of the 3 interventions

	Before Treatment n=41	After Treatment n=41	p values
Glibenclamide treatment			
FPG (mg/dl)	199.54±94.62	156.20±66.90	0.001
PPG (mg/dl)	261.24±113.97	194.63±84.99	0.001
Extract Treatment			
FPG (mg/dl)	197.27±89.70	181.20±76.02	0.037
PPG (mg/dl)	264.80±116.65	238.93±98.80	0.024
Combination Treatment			
FPG (mg/dl)	204.73±93.96	154.54±69.19	0.001
PPG (mg/dl)	267.24±111.73	208.32±89.45	0.001

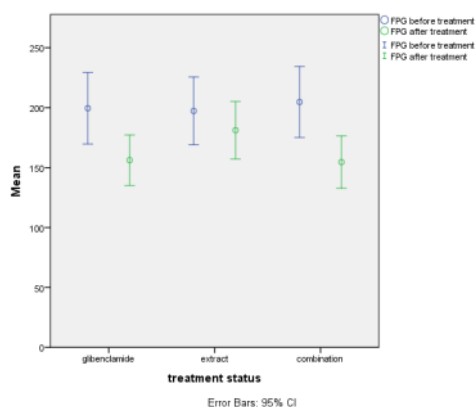


Fig. 2. Changes in Plasma Glucose within treatment groups.

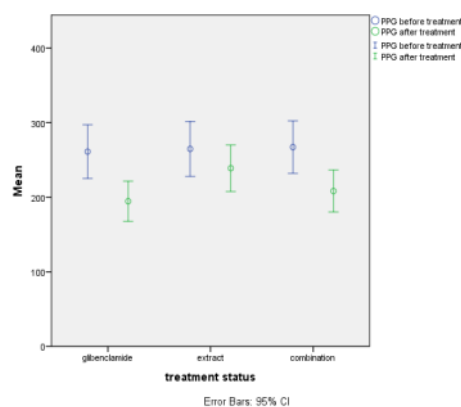
Combination treatment group was most effective in lowering FPG (50.20±51.72mg/dl), followed by Glibenclamide groups (43.34±53.62mg/dl) then Extract treatment (16.07±47.77mg/dl). Meanwhile for lowering PPG, glibenclamide group is the best (66.61±54.99mg/dl), followed by Combination treatment (50.47±66.86mg/dl) and in the last was Extract groups (25.88±70.68mg/dl)(Table 3). The standard deviation were of wide range because of very wide FPG and PPG range at baseline as well as due to extensive range in plasma glucose as determined in inclusion criteria to meet the minimal number of subjects (Figure 3).

Table 3: The differences of FPG and PPG before and after treatment within treatment groups

	Difference n=41	p values
FPG (mg/dl)		
Glibenclamide treatment	43.34±53.62	0.007
Extract Treatment	16.07±47.77	
Combination Treatment	50.20±51.72	
PPG (mg/dl)		
Glibenclamide Treatment	66.61±54.99	0.013
Extract Treatment	25.88±70.68	
Combination Treatment	50.47±66.86	

3 4. Discussion

According to our knowledge the present study is the first Randomized Clinical Trial that reports the effectiveness



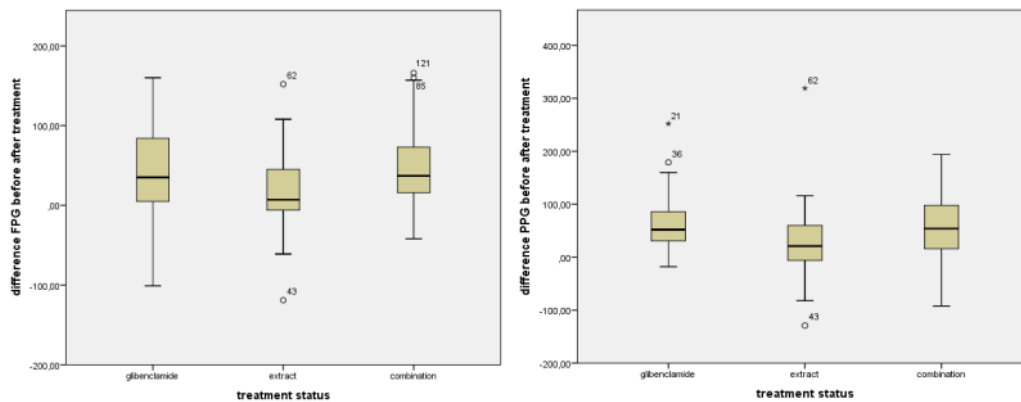


Fig. 3. Plasma glucose difference within treatment groups.

of herbal treatment of mixture extract *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata* on hypoglycemic effect. A single month treatment of mixture herbal extract was found to be effective in lowering FPG in T2DM, however the differences of lowering FPG is less than a single month treatment of glibenclamide or combination of both extract and glibenclamide treatment. The differences were significant though lowered narrowly. This finding strengthens our previous pre clinical study that found mixture extract *Pterocarpus indicus*, *Momordica charantia*, *Phaseolus vulgaris* and *Andrographis paniculata* at 27 mg/200 gBW in a diabetic mice model⁹. This dose is equivalent to 1.5 g in human adult or equal to 22mg/kgBW⁹. Interestingly in this recent study also it was found that combination therapy was the most effective therapy in lowering FPG.

Our recent study found that a single month treatment of mixture extract to be effective in lowering PPG in T2DM patients as well. The differences of lowering FPG in extract group was less than a single month treatment of glibenclamide nor combination of both extract and glibenclamide treatment. This finding also strengthens the finding in several pre clinical studies that suggested effect of extract both in a single or mixture extract on hypoglycemics effect^{7,11,12}. However the standard deviation was having a very large range, because of the selection of the subject included in the study, further investigation needed involved T2DM patients with almost homogenous FPG and PPG at base line.

Our finding also was in line with previous clinical trial, a comparative trial of combine administration *Momordica charantia* extract juice with diet treatment compare with just diet treatment. This previous study found that administration of extract was effective to lowering FPG, HbA1C and serum Sialic Acid (SSA) as well. This study did not find the effectiveness of lowering total cholesterol, HDL-cholesterol, LDL-cholesterol¹³⁻¹⁵. Our recent study did not perform the effect of extract to HbA1C level and SSA because the study was only conducted in a month, that would effect no differences on such parameters.

Recent study was also in line with previous study which investigated the effect of *Pterocarpus sp.* that showed evidence of lowering blood glucose in diabetic rat model¹⁶⁻¹⁸. Clinical trial of mixture extract that consists of *Pterocarpus sp.* extract was done as well and the results shown significantly reduce FPG from 180 ± 10.5 mg/dl to 130 ± 5.5 mg/dl and PPG shown reduce from 200 ± 22.5 mg/dl to 140 ± 13.5 mg/dl as well. This study has also found that no adverse reaction occurred in three months prospective study¹⁹. Our recent study was conducted for a short time period of prospective investigation. Although we observed some clinical sign and symptoms, we were not satisfied to draw a conclusion. Previous study was also investigated the effect of *Phaseolus vulgaris* in lowering blood glucose in diabetic rats²⁰. Other study also showed the positive effect of *Andrographis paniculata* to reduce blood glucose in diabetic rats model as well⁸. Recent study

enhanced these findings, however further study is needed to confirm possibility of adverse clinical effects.

5. Conclusion

The mixture extract of *Pterocarpus indicus* 20%, *Momordica charantia* 10%, *Phaseolus vulgaris* 40% and *Andrographis paniculata* 30%, well known as Diabmeneer[®] was found to be effective in lowering both FPG and PPG in T2DM patients. Future studies are warranted to further investigate the effects of Diabmeneer[®] on glycemic control in T2DM patients, which involves a detailed biomolecular marker.

6. Acknowledgment

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7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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