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RESEARCH ARTICLE

Antihyperglycemic effects of *Cajanus cajan* L. (pigeon pea) ethanolic extract on the blood glucose levels of ICR mice (*Mus musculus* L.)

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ABSTRACT

Background: Diabetes mellitus is a metabolic disease that is usually associated with abnormalities in the metabolism of different substrates leading to high blood glucose levels (BGL). **Aims and Objectives:** Aims and objectives are to evaluate the effects of ethanolic extracts of *Cajanus cajan* (CCEE) leaves on its antihyperglycemic effects on BGL of normoglycemic mice. **Materials and Methods:** 45, 5-week-old female, mice were divided into five groups and were administered with either double distilled water, glimepiride, or CCEE. **Results:** Baseline BGLs were measured before the induction of hyperglycemia by glucose loading. 30 min after, the BGLs were measured again and ranged between 167.667 and 185.778 mg/dL. Immediately after the hyperglycemic BGLs were measured, the corresponding treatments were administered. Subsequently, three blood collections were done spanning 3 h with 1 h interval. The treatment doses were observed to have a faster onset than the positive control supported by the fact that the treatment groups had significant differences ($P < 0.05$) with their respective hyperglycemic BGLs on the 1st h, while the positive control group exhibited significant difference with its hyperglycemic BGL on the 2nd h. The hypoglycemic effect of the positive control peaked at the 3rd h with 67.33 mg/dL and surpassed the hypoglycemic effect of the medium- and high-dose groups with 83.778 and 98.556 mg/dL, respectively. The low-dose group did not have significant difference with the negative control group past the 1st h suggesting that this dosage has a weak hypoglycemic effect. **Conclusion:** These results indicate that the CCEE leaves were able to produce hypoglycemic effects at doses between 400 and 800 mg/kg bodyweight.

KEY WORDS: Antihyperglycemic; Blood Glucose; *C. cajan; Mus musculus*; Oral Glucose Tolerance Test

INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine disease that involves abnormalities in the body's metabolism of carbohydrates, proteins and fats, which are all characterized by high blood glucose levels (BGL) also known as hyperglycemia. This usually results from defects in insulin production, insulin action or both.[1] As of the 2015 International Diabetes Federation Diabetes Atlas (7th edition), it was estimated that 415 million people in the world have diabetes, and this was projected to increase to 642 million people by 2040.[2] On the other hand, based on the 2008 report of the Food and Nutrition Research Institute of the Department of Science and Technology, the prevalence of diabetes in the Philippines has increased to 4.8% from 3.4% in 2003.[3]

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In the Philippines, synthetic medications are often inaccessible to the majority of the population due to its high cost. For example, the average annual income of a Filipino family, adjusting for inflation, is estimated to be around US $3,765.00,[4] whereas a single antidiabetic drug glimepiride

is priced at US $40.00. Therefore, there is a great need to develop cheap alternative supplements for DM.

Cajanus cajan, also known as pigeon pea or "kadyos" (Filipino) is a local legume plant that has been used traditionally as functional food.[5] Sharma et al.[6] investigated the nutritional and medicinal properties of *C. cajan*. It was observed that it is rich in starch, protein, calcium, manganese, crude fiber, fat, trace elements, and minerals. Besides its nutritional value, it also possesses various medicinal properties due to the presence of phenol, tannin, lignins, glycosides and steroids, flavonoids, alkaloids and terpenoids in ethanol extract,[7] all of which have beneficial effects to human health. The leaves of *C. cajan* have been widely used in different countries as a treatment for the treatment of wounds, aphtha, bedsores, malaria, diabetes, diet-induced hypercholesterolemia and to kill worms,[8] ulcer, wound, asthma,[9] and diarrhea and anemia.[5] Radical-scavenging assays confirmed the antioxidant activity of ethanolic extracts of *C. cajan* (CCEE).[8] The anticancer activity of cajanol toward human breast cancer cells-7 was investigated. It was observed that cajanol arrested the cell cycle in the G2/M phase and induced apoptosis through a reactive oxygen species-mediated mitochondria-dependent pathway.[10] *C. cajan* methanolic leaves and stems have been used for the treatment for jaundice[11] and diabetes.[11-13] However, since methanol has been considered to be more toxic compared with ethanol, the study will try to evaluate the effects of CCEE in normoglycemic mice. This study aims to determine the effect of the use of CCEE in lowering the BGL postprandial in normoglycemic mice.

MATERIALS AND METHODS

Preparation of CCEE Leaf

Fresh mature leaves from *C. cajan* shrubs were collected from Commonwealth Avenue, Quezon city. The plant was authenticated by Danilo N Tandang, Museum Researcher II, Botany Division at the National Museum of the Philippines. The leaves were removed from the branches, washed, and then weighed. These were lyophilized using a cascade-type freeze dryer in the Chemistry Department of De La Salle University (DLSU). Using an electric grinder, the freeze-dried leaves were powdered and then soaked in 95% ethanol for 48 h in an amber bottle before decantation. The extract was processed using Soxhlet apparatus to remove the ethanol. The plant extracts were stored in an amber bottle container at room temperature before use.

Test Animals

45, 4-week-old, 20-30 g female, Institute for Cancer Research (ICR) mice from Research Institute for Tropical Medicine, Alabang, Muntinlupa city were purchased. The mice were housed in the DLSU Biology Animal House placed in 5 × 6 × 5 in plastic cages with metal mesh tops. Soft wood shavings were used as beddings and were replaced weekly. The animals were fed pellet diet and water *ad libitum.* All the animals were acclimatized for 7 days into adjust to a 12 h light: 12 h dark cycle at 24 ± 2°C and relative humidity of 55 ± 10% conditions. Before the experimentation, baseline glucose levels were measures to ascertain that none of the mice have high BGL, all of which were within the normal limits before the experiment proper.

Treatment Solution Preparation

The glucose solution, plant extract solution, and the glimepiride solution were prepared fresh on the day of the experiment. The glucose solution was prepared by dissolving 20 g of D-glucose powder in 80 mL of double distilled water and then it was vortexed until the solution became homogenous. This working solution had a 250 mg/mL concentration. The plant extract solution was prepared by mixing 1 g of plant extract in 10 mL of double distilled water and then it was vortexed until the solution was homogenous. This working solution had a 100 mg/mL concentration. Finally, the glimepiride solution was prepared by mixing 2 pieces of 2 mg tablets in 8 mL of double distilled water and then was vortexed until the solution was homogenous. This working solution had a concentration of 0.5 mg/mL.

Experimental Procedures

The animals were allowed to fast for 24 h before treatment administration. On the day of the experiment, the mice were divided into five groups consisting of 9 individuals each (Table 1).

All the animals were weighed to determine the amount of treatment to be administered for the duration of the experiment. Their weights ranged from 20 to 30 g. The experiment was done in batches starting from the controls, then the low dose, medium dose and the high dose. At the start of each group, the baseline BGL of each mouse was measured using the EasyTouch® glucose cholesterol uric acid (GCA) meter. The blood was collected from the tail vein via a small transection near its tip. Blood collection for the duration of the experiment was taken from the same transection unless it has fully clotted already; only then was

Table 1: Treatment groupings

Group	Treatment
Negative control group	0.2 mL of double distilled water
Positive control group	16.77 µg/kg body weight standard drug glimepiride
Low dose group	200 mg/kg body weight of plant extract
Medium dose group	400 mg/kg body weight of plant extract
High dose group	800 mg/kg body weight of plant extract

a new transection done nearer the base of the tail. After blood collection, the wound was dabbed with sterile cotton swabs to stop the bleeding. After the bleeding has stopped, the glucose solution was orally administered with a dose of 5 mg/g bodyweight. After exactly 30 min from the administration of the glucose solution, blood was collected and measured using the GCU meter, and then the designated treatment was immediately administered via oral gavage. After 1 h from the administration of glucose, the blood was again collected and measured using the GCA meter. This was repeated two more times at 1 h interval.[14] The procedures used in this study was approved by the DLSU Institutional Animal Care and Use Committee (2015-004) following recommendations of the Philippine Association for Laboratory Animal Science. The experiment from extraction to administration of samples was performed from January to March 2015.

Statistical Analysis

All data will be expressed as mean ± standard deviation (SD). Statistical differences between means within groups were evaluated using repeated measures analysis of variance (ANOVA), and statistical differences within time intervals and means were evaluated using multivariate ANOVA followed by Tukey's test using Statistical Packages for the Social Sciences version 22. $P < 0.05$ was considered significant.

RESULTS

Three different doses of *C. cajan* extract (200, 400, and 800 mg/kg), glimepiride as the positive control (16.7 µg/kg), and distilled water as the negative control were orally administered to 45 ICR mice (*Mus musculus*). Five (5) blood collections were done to quantify BGLs (mg/dL) spanning 3 h (Figure 1 and Table 2).

Post hoc tests showed that there were no significant differences among baseline BGLs. They also fall within the established normal range of BGL for mice.[15] This suggests that all of the mice were healthy at the start of the experiment. After the baseline measurements were taken, glucose was administered.

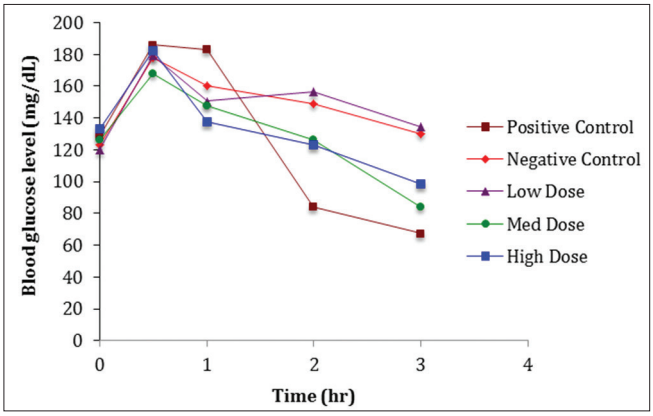

Figure 1: Effect of graded doses of the plant extract on blood glucose levels in Institute for Cancer Research mice over a 2.5-h period

Half an hour (0.5) after the administration of the glucose, blood from the mice were again collected and measured. All of the groups exhibited hyperglycemia, and no significant difference was observed across all groups. After the measurements at the ½ h were taken, the corresponding treatments were administered.

On the 1st h of the experiment, the treatments have had 30 min to take effect. The positive control group still exhibited hyperglycemia. The BGL of the *C. cajan* extract groups significantly decreased ($P < 0.05$), and had no significant difference with each other, but was still over baseline BGL. The negative control group also exhibited hyperglycemia and however was not significantly different ($P < 0.05$) to the plant extract groups.

On the 2nd h of the experiment, the treatments have had 1½ h to take effect. The positive control group now exhibited hypoglycemia and was significantly different ($P < 0.05$) across all groups. This was followed by the medium- and high-dose groups, which had no significant difference ($P < 0.05$) with each other, and was now around their corresponding baseline BGL. The low dose and negative group had the highest BGL among the groups and were also still slightly hyperglycemic.

On reaching the 3rd h of the experiment, the positive control group had reached a mean BGL of 67.333 ± 13.02, which was significantly lower ($P < 0.05$) than all the other groups. The medium- and high-dose groups were slightly hypoglycemic and expressed no significant difference ($P < 0.05$) with each other. Finally, the BGL of the low dose and negative control groups has reduced back to around their corresponding baseline BGL.

Among the groups, the negative control showed the slowest reduction in BGL. This was consistent with expected results because the negative control, which only contained water, was used to demonstrate the natural normalization of the BGL of the mice. Thus, the varying rate of reduction of BGL of the other groups can be attributable to the treatment that was administered to each, respectively.

The results showed that the CCEE leaves had significant hypoglycemic effect when administered at doses of 400-and 800 mg/kg body weight. However, the lowest dose (200 mg/kg) of the plant extract did not have a strong hypoglycemic effect supported by the fact that the low-dose group had no significant difference ($P < 0.05$) with the negative control group past the 1st h. Notably, the medium and high dose took effect faster than the standard drug, glimepiride.

Table 2: Effect of graded doses of ***C. cajan*** ethanolic leaf extract on BGL in ICR mice

Group	Baseline	0.5 h	1 h	2 h	3 h
Negative control	123.333±9.71[a; 4]	177.778±22.40[a; 1]	160.444±17.84[a, b; 1,2]	149.000±20.69[a; 2,3]	130.000±11.90[a; 3,4]
Positive control	123.778±15.33[a; 2]	185.778±31.79[a; 1]	183.000±30.72[a; 1]	84.222±9.59[c; 3]	67.333±13.02[c; 3]
Low dose	120.222±12.66[a; 4]	179.111±22.86[a; 1]	150.444±16.50[b; 2,3]	156.444±13.70[a; 2]	134.444±15.74[a; 3,4]
Medium dose	126.000±17.98[a; 2]	167.667±41.18[a; 1]	147.556±28.12[b; 1,2]	126.222±14.05[b; 2]	83.778±9.86[b; 2,3]
High dose	133.111±18.48[a; 2]	182.333±34.26[a; 1]	137.333±15.99[b; 2]	123.111±11.56[b; 2,3]	98.556±7.80[b; 3]

The mean±SD values with the same numerical superscripts within rows are not significantly different (P<0.05). The mean±SD values with the same letter superscripts within columns are not significantly different (P<0.05). SD: Standard deviation. BGL: Blood glucose levels, ICR: Institute for Cancer Research

The effects of positive control group were first observed at the 2nd h of the experiment. At this point, the glimepiride has had 1½ h to take effect. It peaked 2½ h after the administration. This is consistent with the studies that show significant absorption of glimepiride occurs within 1 h and peaks at 2-3 h (Sanofi-Aventis, 2013).

DISCUSSION

Phytochemical constituent investigations have indicated that *C. cajan* leaves contain flavonoids, saponins, and tannins.[7] Flavonoids are secondary plant metabolites that are well known for their antidiabetic activity among other biological activities. They suppress BGL, reduce plasma cholesterol and triglycerides and increase hepatic glucokinase activity by enhancing the insulin release from pancreatic beta cells.[16] The isolated flavonoid compounds, 4-hydroxybenzaldehyde, myricetin-3-O-rham-noside, europetin-3-O-rhamnoside, phloretin, myrigalone-G, and myrigalone-B were studied for their antidiabetic activity. In this study, all of these compounds were seen to inhibit alpha-glucosidase and alpha-amylase activity better than the antidiabetic drug, acarbose. In addition, phloretin was shown to reduce insulin resistance by adipocyte differentiation and adiponectin secretion.[17,18]

Saponins are glycosylated compounds with natural detergent activity, which are found in *C. cajan* leaf extracts. They are known to lower BGL by stimulating insulin secretion from pancreatic beta cells increasing the utilization of peripheral glucose. They are classified into triterpenoid, steroid or steroidal glycoalkoid.[19] A study conducted by Chen et al.[20] investigated the antidiabetic effects of isolated saponins on KK-Ay mice. The mice were administered the plant extracts in 50 or 200 mg/kg bodyweight dosages. Their results showed the improvement in glucose tolerance, reduction insulin resistance indices and triglyceride levels of the treatment groups, and the development of the mice glomular lesions was prevented due to the ability of the saponins to promote the regeneration of pancreatic beta cells and inhibition of hepatic gluconeogenesis.

Tannins are polyphenolic secondary metabolites of higher plants that are speculated to have the ability to bind to carbohydrates, leading to the formation of indigestible complexes.[21] The antidiabetic activity of tannins was evaluated with porcupine pancreatic amylase, and the inhibitory effect of the alcoholic extracts was found to be comparable with the antidiabetic drug, aracbose.[22] The antihyperglycemic effect was attributed to the ability of tannins to inhibit the alpha-amylase and to bind to carbohydrates leading to the formation of complexes that cannot be absorbed.

In this study, saponins, flavonoids and tannins known to have hypoglycemic effects may be inferred to synergistically contribute to the antidiabetic property of *C. cajan* leaf extract.

CONCLUSION

The CCEE leaves were able to lower the BGLs of ICR mice at doses of 400 and 800 mg/kg of body weight. Furthermore, the treatment groups had a faster onset than the positive control group as evidenced by the values during the 1st h. The low-dose group did not have significant difference with the negative control group past the 1st h suggesting that this dosage of the plant extract only has a weak hypoglycemic effect. On the 2nd h, the positive control produced the strongest hypoglycemic effect among the groups, which peaked at the 3rd h. Notably, the medium-dose group had a lower blood glucose mean (83.778 ± 9.86) than the high-dose group (98.556 ± 7.80) albeit not significantly different.

Saponins, flavonoids, and tannins found in *C. cajan* leaves as reported by related literature could have synergistically contributed to its antihyperglycemic effects. Weaker hypoglycemic effects were noted from the low-dose group. The results of the medium dose and high dose were statistically similar of having a strong hypoglycemic effect. This indicates that the optimum dose of CCEE leaves ranges from 400 to 800 mg/kg bodyweight.

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