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*Full Length Research Paper*

# Screening for antidiabetic activity and phytochemical constituents of common bean (*Phaseolus vulgaris* L.) seeds

**A. L. Ocho-Anin Atchibri[1]*, K. D. Brou[1], T. H. Kouakou[2], Y. J. Kouadio[2] and D. Gnakri[1]**

[1]Université d'Abobo-Adjamé, UFR Sciences et Technologie des Aliments, Laboratoire de Nutrition et de Sécurité Alimentaire, 02 BP 801 Abidjan 02, Côte d'Ivoire.
[2] Université d'Abobo-Adjamé, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire.

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**Seeds of *Phaseolus vulgaris* were given individually at different doses to different batches of rats (both normal and hyperglicemic rats) after an overnight fast. Seeds contain the bioactive components – alkaloids, flavonoids, fiber, proteins, tannins, terpenoids, saponins, quercetin, anthocyanin and catechin. The blood glucose levels were measured at 0, 1, 2, 3, 4, 5 and 6 h after the treatment. Most active doses were further studied to dose-dependent (300, 200 and 100 g/kg bw) antihyperglycemic effects alone and in combination with glibenclamide (0.20, 0.10 and 0.05 g/kg bw). Seeds of *P. vulgaris* at a dosage of 300 g/kg bw is showing maximal blood glucose lowering effect in diabetic rats after third hour. The antihyperglycemic activity of *P. vulgaris* seeds was compared with the treatment of glibenclamide, an oral hypoglycemic agent. The combination of seeds of most dose (300 mg/kg bw) and higher dose of glibenclamide (0.20 g/kg bw) showed safer and potent hypoglycemic as well as antihyperglycemic activities without creating severe hypoglycemia in normal rats.**

**Key words:** *Phaseolus vulgaris*, seeds, diabetes mellitus, antihyperglycemic activity, glucose-infused diabetes, glibenclamide.

## INTRODUCTION

The origin and domestication of common bean (*Phaseolus vulgaris*) has been established in America (Papa et al., 2005). Dry common bean is a legume widely consumed throughout the world and it is recognized as the major source of dietary protein in many Latin-American and African countries (Guzman-Maldonado and Paredes-Lopez, 1998). Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. These metabolic disorders include alterations in the carbohydrate, fat and protein metabolisms associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus and unexpected weight loss, etc. There is an increasing demand by patients to use the natural products with antidiabetic activity, due to the side effects associated with the use of insulin and oral hypoglycemic agents (Botero and Wolfsdorf, 2005). In 2010, according to World Health Organisation (WHO), 221 million people in the world were diabetic. In Africa, diabetes has a rapidly and was currently regarded as a public health problem (N'guessan, 2008; Konkon et al., 2010). World Health Organization estimated that there are 14 million people with diabetes in Africa in 2000, which is projected to rise to 30 million by the year 2025. Globally, the number of people that has been diagnosed with diabetes has exploded in the past two decades. With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amounts of

*Corresponding author. E-mail: aninocho@yahoo.fr. Tel: (+225) 07 14 76 77 / 20 30 42 79. Fax: (+225) 20 30 43 42.

resources including medicines, diets, physical training, and so on in all countries. Therefore, it is very important to search new therapeutic strategies which might be cheaper, safe, and convenient for treatment of diabetes.

In Africa, use of plant medicines is very common practice from ancient time, and it is considered as much safer and less expensive therapeutic strategies for treatment of various diseases (Ambady and Chamukuttan, 2008). The use of plant medicines for treatment of diabetes has been reported long ago. There is plethora of literature that is available for antidiabetic plants (Modak et al., 2007; Carai et al., 2009; Mishra et al., 2010; Konkon et al., 2010. Pravin (2006) reported that about 70% of the human population is dependent (wholly or partially) on plant-based medicines and the World Health Organisation estimates that 80% of the world population presently uses herbal medicine for some aspect of primary health care (WHO, 2008). The potential of medicinal plants research results in health care is no longer in doubt, having gained recognition in several nations of the world.

The available literature shows that there are more than 400 plant species showing hypogly-cemic activity (Ivorra et al., 1989; Konkon et al., 2010). Though some of the plants are reputed in the indigenous systems of medicine for their activities, it remains to be scientifically established. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken (WHO, 1980). *P. vulgaris* have a notable place in the folklore throughout the world and in the traditions of many cultures such as antidiabetic activity (Tormo et al., 2004; Carai et al., 2009; Mishra et al., 2010).

Preclinical investigations have unanimously reported how the acute, repeated administration of extracts of *P.* vulgaris, as well as some of their isolated ingredient reduced food intake, body weight and lipid accumulation in lean and obese laboratory animals have been carried out on this plant. Although, a surfeit of literature is available for the antidiabetic potential of *P. vulgaris,* the selected seeds (which are well-known as a good dietary source) are an interesting pharmacological activity according to several authors (Hangen and Bennink, 2002; Helmstädter, 2010). Currently, the data on antidiabetic properties and bioactive constituent of *P. vulgaris* seeds are limited in literature. Hence, the present study was undertaken to determine the chemical constituent and investigate the antihyperglicmic effects of *P. vulgaris* seeds.

## MATERIALS AND METHODS

### Plant material

Vegetable material is constituted of common bean (*P. vulgaris*) seeds, which were purchased from Abidjan (Côte d'Ivoire) markets.

### Phytochemical screening

Screening of phytochemical constituents of the plant was done using standard procedures described by several authors (Harbone, 1978; Sofowora, 1984; Trease and Evans, 1996; Adetuyi and Popoola, 2001; Eogona et al., 2005; N'Guessan, 2008; Konkon et al., 2010; Yadav et al., 2010). The extracts obtained with the powder coming to freeze-dried seeds were used to identify and characterize some chemical groups.

### Animals and experimental schedule

Male Wistar rats of 4 - 5 weeks old were housed in polypropylene cages at 22 ± 2°C ambient temperature and 55 ± 5% humidity in 12/12 light and dark cycle. Rats were fed with a maintenance food until a weight of approximately 180 g. Water is provided *ad-libitum* animals and put out in individual cage for the study. A total of 42 male rats were used for the study. This study was completed in two phases. Phase 1: seeds of bean were selected and administered in 12-h-fasted normal animals at 300 g/kg bw. To study antihyperglycemic activity, 200 g/kg bw dose of glucose was administered to 12-h-fasted animals (for induction of hyperglycemia) at the same time of glucose ingestion. Phase 2: The antihyperglycemic effect of bean seeds was studied for dose-dependent effects and its drug interactions with standard antidiabetic medicine, that is, glibenclamide. Three different doses of common bean seeds (300, 200 and 100 g/kg bw) and glibenclamide (0.20, 0.10, and 0.05 g/kg bw) were tested for antihyperglycemic potential.

### Estimation of blood glucose

After administration of seeds and/or standard drug, the blood samples were collected from tail tip, and glucose was monitored using glucometer strips (Roche Diagnostics, Indiana, USA) at 1 h interval for 6 h.

### Statistical analysis

Data were analyzed using Statistical software (release 7.5). Differences in mean values were tested by analysis of variance, and significance levels were obtained with Duncan's test. A significance level of $p < 0.05$ was used. Data are the means of three replicates.

## RESULTS AND DISCUSSION

Basic phytochemical screening comprising chemical tests to detect the presence of alkaloids, anthraquinones, glycosides, polyphenols, saponins, steroids, trepenoid and tannins (Table 1). All compounds analyzed in the seeds were significantly differents. Hyperglycemia is a chronic state of diabetic condition; in fact, chronic hyperglycemia is the defining characteristic of the disease. The pathophysiology of hyperglycemia in diabetic state is very complicated and affects by many daily activities such as food intake, exercise, etc. Long-term hyperglycemia causes several microvascular and macrovascular complications of diabetes (Brindisi et al., 2006; Calcutt et al., 2009). Therefore, the control of hyperglycemia needs special attention in diabetic conditions. Although, there

**Table 1.** Phytochemical analysis of *Phaseolus vulgaris* seeds

| Active principle | Extract |
|---|---|
| Alkaloids | + |
| Anthraquinone | ++ |
| Catechic tannins | +++ |
| Flavonoids | ++++ |
| Gallic tannins | + |
| Glycosides | ++++ |
| Polyphenols | +++ |
| Saponins | +++ |
| Steroids | + |
| Terpenoids | + |

(+) Present; (-) Absent.

are various oral hypoglycemic regimens are available in market, but conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand, plant extracts are expected to have similar efficacy without side effects as that of conventional drugs. Hence, in present study, we evaluated the antihyperglycemic potential of P. vulgaris seeds. After a thorough reviewing the literature on antidiabetic effects of P. vulgaris, it has been found that various studies reported the effect of different part of this plant in different diabetic models (Carai et al., 2009 ; Tormo et al., 2004; Mishra et al., 2010; Yadav et al., 2010).

According some authors, *P. vulgaris* is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals which have a potential benefits on health (N'guessan, 2008; Mishra et al., 2010). Important biological activities have been described from common beans like enhancement of the bifidogenic effect (Queiroz-Monici et al., 2005); anti-oxidant (Heimler et al., 2005); anticarcinogenic (Hangen and Bennink, 2002) effects. Interestingly, we found that all doses of *P. vulgaris* seeds were active for antihyperglycemic potential. The reason for this active antihyperglycemic activity of *P. vulgaris* seeds might be due to their availability phytoconstituents, that is, alkaloids, flavonoids, tannins, terpenoids and saponins (Henningson et al., 2001; Sharma et al., 2003). It has also been reported earlier that terpenoids (Murakami et al., 2001), saponins (Kambouche et al., 2009), are precise bioactive components responsible for the antidiabetic activity of *P. vulgaris* seeds. In addition, we also studied the combination of most bioactive dose of *P. vulgaris* seeds with standard drug; glibenclamide was also investigated to find out how much dose of its can be reduced by combining most active dose (Table 2). This part of study shows very interesting results that the combination of most active dose of *P. vulgaris* seeds significantly reduced the dose of glibenclamide (from 0.20 to 0.05 g/kg bw) in hyperglycemic animals, and it was safer in normal rats also, while combination with higher dose (0.20 g/kg bw) was chronic to produce hypoglycemia in normal rats. The antihyperglycemic effects of *P. vulgaris* seeds might be either stimulating pancreatic B cells to secrete more insulin (insulin secretor) or increased insulin sensitivity in peripheral tissues, that is., adipose tissue, muscle, and live to clear blood glucose at faster rate. The exact mechanism of action of *P. vulgaris* seeds is not well-known, but some authors give tracks of responses. Literature suggests the involvement of two possible mechanisms of action in the reducing effect of *P. vulgaris* extracts on glycemia. Both these mechanisms focus on the role of phytohemo-agglutinin and α-amylase inhibitors. Pancreatic α-amylase is an enzyme that catalyzes hydrolysis of α-(1,4)-glycosidic bonds of starch polymers (Santimone et al., 2004). Thus, inhibition of α-amylase results in the suppression of starch metabolism and, in turn, a decrease in glycemia (Ishimoto et al., 1995). It has also been reported that $\alpha$-amylase inhibitors delay gastric emptying, producing feelings of satiety (Jain et al., 1991), thus resulting in reduced food intake (Tormo et al., 2006).

Phytohemoagglutinin is known to bind to the stomach epithelial cells and to the brush border membrane of small intestine, cecum, and colon (Herzig et al., 1997). This binding results in the stimulation of the release of cholecystokinin and glucagonlike peptides (King et al., 1986; Radberg et al., 2001), two hormones playing a relevant role in digestive processes. In close agreement with the latter hypothesis, recent data indicate that treatment with the cholecystokinin receptor type A phyto-hemoagglutinin is the stimulation of pancreatic secretion of α-amylase in rats (Baintner et al., 2003); this should result in an accelerated metabolism of ingested starch and, in turn increase in glycemia. Nevertheless, based on these results, it may be speculated that most active *P. vulgaris* seeds had similar activity as glibenclamide as an insulin secretor in hyperglycaemic rats. Together, these data suggest that extracts of *P. vulgaris* seeds may constitute potentially interesting, novel remedies for the treatment of metabolic syndrome such as diabetes. Physiological impact of *P. vulgaris* seeds on post-ingestif glucose could be integrated in strategies of treatment and the prevention. In the preceding strategies, the consumption of glucides is often limited, but deliberated selection of foodstuffs with low glycemic index is a more modern approach with the problem.

## Conclusion

From this study, it may be concluded that the *P. vulgaris* seeds have antihyperglycemic potential and may use as complementary medicine to treat the diabetic population by significantly reducing dose of standard drugs. They may suggest that the combination of most active dose of

**Table 2.** Effect of *Phaseolus vulgaris* seeds and glibenclamide on fasting blood glucose levels (mg/dl) of normal and diabetic rats.

| Groups | Blood glucose at different hours after the treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| **Normal rats** | | | | | | | |
| Seeds | | | | | | | |
| 300 g/kg bw | 80 [a] | 110 [c] | 130 [f] | 100 [b] | 85 [a] | 74 [n] | 64 [g] |
| 200 g/kg bw | 81 [a] | 100 [b] | 125 [df] | 87 [a] | 82 [a] | 71 [n] | 65 [g] |
| 100 g/kg bw | 84 [a] | 115 [c] | 120 [d] | 89 [a] | 81 [a] | 78 [j] | 68 [g] |
| *Glibenclamide* | | | | | | | |
| 0.20 g /kg bw | 87 [ab] | 108 [c] | 124 [de] | 85 [a] | 81 [a] | 74 [n] | 63 [g] |
| 0.10 g/kg bw | 85 [a] | 105 [bc] | 127 [f] | 85 [a] | 80 [a] | 75 [n] | 66 [g] |
| 0.05 g/kg bw | 86 [a] | 112 [c] | 125 [df] | 89 [a] | 85 [a] | 80 [n] | 69 [g] |
| Seeds + glibenclamide | | | | | | | |
| 300 + 0.20 g/kg bw | 85 [a] | 70 [e] | 60 [g] | 48 [k] | 35 [m] | 25 [b] | 08 [c] |
| 300 + 0.10 g/kg bw | 80 [a] | 77 [ae] | 68 [h] | 40 [l] | 33 [lm] | 22 [b] | 12 [c] |
| 300 + 0.05 g/kg bw | 82 [a] | 79 [a] | 65 [h] | 4l [f] | 37 [n] | 40 [f] | 45 [f] |
| **Hyperglycemic rats** | | | | | | | |
| Seeds | | | | | | | |
| 300 g/kg bw | 94 [b] | 120 [d] | 138 [e] | 130 [il] | 128 [l] | 123 [d] | 121 [d] |
| 200 g/kg bw | 90 [b] | 117 [d] | 135 [ei] | 133 [ei] | 131 [l] | 127 [l] | 123 [d] |
| 100 g/kg bw | 89 [b] | 118 [d] | 133 [ei] | 130 [ei] | 127 [gl] | 125 [dl] | 120 [d] |
| Glibenclamide | | | | | | | |
| 0.20 g /kg bw | 90 [b] | 130 [f] | 145 [j] | 140 [j] | 135 [f] | 130 [f] | 125 [f] |
| 0.10 g/kg bw | 93 [b] | 132 [f] | 137 [ij] | 144 [i] | 139 [f] | 133 [f] | 130 [f] |
| 0.05 g/kg bw | 92 [b] | 127 [fg] | 136 [j] | 146 [j] | 143 [j] | 139 [fj] | 135 [f] |
| Seeds + glibenclamide | | | | | | | |
| 300 + 0.20 g/kg bw | 93 [b] | 125 [d] | 112 [c] | 115 [c] | 123 [d] | 120 [d] | 122 [d] |
| 300 + 0.10 g/kg bw | 94 [b] | 120 [d] | 118 [c] | 110 [c] | 124 d | 126 [l] | 121 [d] |
| 300 + 0.05 g/kg bw | 90 [b] | 124 [d] | 111 [c] | 113 [c] | 121 [d] | 123 [d] | 120 [d] |

Values are means six animals in each group. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$.

*P. vulgaris* seeds with glibenclamide may play an important role to reduce the blood glucose levels in chronic diabetic conditions. Moreover, further study is required, pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic effect of *P. vulgaris* seeds. In the same way, it could be interesting to carry out isolation, purification and characterization of bioactive active components from seeds, which might pave a good independent and/or complementary regiment for the treatment of diabetes mellitus, seem to be necessary.

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