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In vitro evaluation of α-amylase inhibitory activity of some medicinal plants used in treatment of diabetes mellitus in Algeria and their effect on postprandial hyperglycemia in normal rats

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Abstract

Postprandial hyperglycemia is an early defect of type 2 diabetes, it is responsible of secondary complication which can affect many organs: heart, kidney, nervous system, and impaired their function. In this type of diabetes mellitus, the inhibition of digestive enzymes (-amylase and -glucosidases) is a useful treatment to attenuate postprandial hyperglycemia.

In this study we investigate *in vitro,* the -amylase inhibitory potential of aqueous extract of leaves or roots of five selected plants recommended to treat diabetes in traditional Algerian medicine. They are also tested for their effect on reduction of postprandial hyperglycemia induced by starch loading in normal rats. The plant extracts showed a variable degree of inhibition of -amylase. The most active sample is the aqueous extract of *Phylleria angustofolia* (PaE) with an IC_{50}=0.61mg/ml followed by extract of *Olea europea* (OeE), *Juniperus oxydrus* (JoE), *Olea europea* var. *Sylvestris* (OsE) and *Salvia officinalis* (SoE). Acarbose (Acb), a standard inhibitor, exhibited an IC_{50} value of 0.07mg/ml. In an animal study, two plant extracts and acarbose exhibited an anti-hyperglycemic activity: *SoE* and *PaE* suppress significantly postprandial hyperglycemia response induced by starch loading in rats, as shown by the significant attenuation of the value of $AUC_{0\text{-}180min}$ by 60 ($p<0.05$) for PaE, 48 ($p<0.05$) for SoE and34 ($p<0.05$) for Ac, compared to control group.

These findings suggest that among the five medicinal plants studied, *Phylleria angustifolia* and *Salvia officinalis* exert their antidiabetic effect by inhibition the digestion of complex carbohydrates, retarding glucose absorption and hence suppress postprandial hyperglycemia.

Keywords: type 2 diabetes mellitus, α-amylase inhibition, postprandial hyperglycemia, medicinal plants.

Introduction

Postprandial hyperglycemia is an early detected symptom in types 2 diabetes, which occurs when pancreatic β cells fail to secrete a sufficient amount of insulin [1]. It is well established that it is one of the risk factor in the development of diabetes and its complications such as cardiovascular diseases and an independent risk factor for atherosclerosis[2]. It has also been implicated in inducing oxidative stress [3].That is recognized as a major pathophysiological link between cardiovascular diseases and diabetes [4]. Therefore, control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of micro- and macro-vascular complications by decreasing of blood glucose rise after food intake. Postprandial hyperglycemia strongly depends on the amount of absorbed monosaccharide and the velocities of absorption in the small intestine. Carbohydrates are recommended to account for a 50% of the daily supply of calories in type 2 diabetes. Monosaccharides play only a minor role as dietary carbohydrate; they consist mainly of complex carbohydrates such as starch and disaccharides, such as sucrose.

Pancreatic amylase is a key enzyme in the digestive system, catalyses the initial step in hydrolysis of starch to produce glucose, maltose, oligosaccharides and dextrins. They are then acted on by -glucosidases, enzymes located at the brush border of the enterocytes, and further degraded to glucose which can be assimilated and transported through the mucosa of the bowel from intestinal lumen to blood circulation. Thus, any medication that reduces or delays breakdown of complex carbohydrates should decrease postprandial hyperglycemia and improve insulin sensitivity, as well as protecting the beta cells of the pancreas[5].

Nowadays, enzymes inhibitors like acarbose, voglibose, and miglitol are current drugs used to control of postprandial hyperglycemia. However, these antidiabetic drugs have undesirable side effects mainly gastrointestinal as abdominal discomfort, diarrhea and flatulence [6].

Plants have been used as the basis of medicines for thousands of years and even today. These traditional medicines are relied upon for health care in many parts of the world [7]. Several medicinal plants species have been used to control diabetes in the traditional medicinal systems of many cultures worldwide [8,9,10]. A number

of them are known to exert their antihyperglycemic activity via the inhibition of carbohydrate hydrolyzing enzymes. Therefore, natural inhibitors from plant sources can offer an attractive strategy for the effective control of postprandial hyperglycemia without or less unwanted secondary effects[11-13].The potential role of medicinal plants as inhibitors of amylase has been reviewed by several authors. A variety of plants has been reported to show amylase inhibitory activity and so many be relevant to the treatment of type 2 diabetes [14,15].

In North Africa countries, many ethnobotanical and ethnopharmacological surveys are conducted to document the indigenous knowledge about healing plants. These studies revealed that about a hundred of plants species are in common use by diabetic patients to treat diabetes mellitus [16-20].

In this study, we investigated the inhibitory potential of five indigenous plants species:*J. oxycedrus* ssp. *Oxycedrus, Salvia officinalis L., Olea europaea L. var.* europaea, *Olea europea* var. *sylvestris* and *Phillyrea angustifolia* (table1), collected in north west area of Algeria. Their crude aqueous extract, prepared according to folkloric usage (decoction), were tested for their inhibitory effect against porcine pancreatic -amylase activity, an enzyme structurally and mechanistically closely related to human pancreatic amylase, with starch as substrate using chromogenic 3,5-Dinitrosalicylic acid method. Plant extracts were also investigated for their effect on postprandial glucose levels in normal rats after starch administration. Their effectwere compared to acarbose, a commercially available α-amylase inhibitor, used as reference.

Material and methods

Chemicals and reagents

Porcine pancreatic -amylase (EC 3.2.1.1) (PPA), 3,5-Dinitrosalicylic acid (DNSA), Soluble potato starch, were obtained from Sigma-Aldrich Chimie GmH, Germany. Acarbose from Glucobay (Bayer, Germany). All other chemical reagents used in this study were of analytical grade.

Plant material

Leaves of *P. angustifolia*, *O. europea*, *O. sylvestris*, *S. officinalis* and roots of *J. oxycedrus* were collected during spring and summer 2013 in the region of Tlemcen in the northwest of Algeria. They were authenticated by a taxonomist at the department of Ecology, University of Tlemcen, Algeria. Voucher specimens were deposited at the Herbarium of the department. Plant materiel were washed and air-dried in the shade and grounded into powder.

Preparation of the Extract

Water extract of each plant were prepared as follow: 100ml of cold water were added to 10g of ground plant materiel and boiled for 15 min. The cooled decoctions were filtered through Whatman No1 filter paper. The filtrates were evaporated to dryness at 40 C and the final solid residues were stored at 4 Cuntil used.

Porcine pancreatic -amylase inhibitory activity

The α-amylase inhibitory activity was determined by assay adapted from method of Bernfeld [21].The reaction mixture contain:200µl of sample test of plant extract at a concentration range of 0.3-3.3mg/ml, 200 µl of 0.02M sodium phosphate buffer(pH 6.9, containing 6.7mMNaCl) containing 1.3U/ml of porcine pancreatic -amylase solution. It was pre-incubated at 37 C for 10min, then 200 µl of 1% starch solution in the above buffer were added and incubated at 37 C for 15min. The reaction were terminated with 600 µl of DNSA color reagent, placed in a boiling water bath for 10 min and cooled down to room temperature. The reaction mixture was then diluted after adding 10 ml of distilled water and the absorbance was measured at 540nm. To eliminate the absorbance produced by plant extract, appropriate extract controls with extract and except the enzyme were also included. Commercial inhibitor acarbose was used as a positive control at a concentration range of 0.033-0.20 mg/ml. Experiments were done in triplicates and the percentage of enzyme inhibition by the sample was calculated according to the formula:

Inhibition of -amylase activity (%)= $[\frac{AbsC - AbsS}{AbsC}]$ 100

AbsC is the absorbance of the control (100% enzyme activity) and AbsS is the absorbance of the tested sample or acarbose. The concentration of an inhibitor required to inhibit 50% of enzyme activity (IC_{50}) under the mentioned assay conditions was determined from plots of log (concentration of extract/acarbose) versus percentage inhibition curves.

Experimental animals

Adult male Wistar rats weighing 200–250 g bred in the animal house of the department of Biology-University of Tlemcen, Algeria, were used in this study. They are maintained under standard laboratory conditions (12h light and dark cycles, 22±2 ◦C and relative humidity of 50–65%) , fed with standard rodent diet and tap water supplied *adlibitum*. The rats were treated in accordance with the universally accepted guidelines for animal experimentation.

Effect of plant extracts on postprandial blood glucose levels following starch loading

Thirty five overnight-fasted normal rats were randomly divided into 7 groups of five each. Group 1(control): received a starch solution at a dose of 5g/kg bw+ water (1ml), administered orally; Group 2,3,4,5,6(experimental groups) were co-administeredorally starch (5g/kg bw) and 250mg/kg bw of plant extract: PaE, OeE, OsE, SoE and JoE respectively; Group 7 (positive control): rats received starch (5g/kg bw) and acarbose (50mg/kgbw).

Blood samples were collected from tail puncture for the measurement of glucose concentration (BGL) immediately before (0min) and at 30,60,120,180min after the starch andtested

extract/acarbose had been administered, using a Glucometer (Accu-Chek, Roche, Germany).

The area under the curve was calculated accounting only for area under curve of incremental blood glucose level during the 180 min test period($AUC_{0\text{-}180min}$), to estimate postprandial glycemic load, on the basis of trapezoidal ruleusing the following formula:

$AUC_{0\text{-}180}$ (g/L.min)=1/2[(a+b)t_1+(b+c)t_2+(c+d)t_3+(d+e)t_4]where a, b, c ,d and eare BGL=(BGL_t-BGL_0) at corresponding time: 0, 30, 60, 120 and 180 min; t_1, $t_2$and $t_3$are the differences between the two consecutive times.

Statistical analysis

All data were expressed as mean±standard error (SEM). Statistical analysis was performed using Student's *t*-test to compare control and treated groups. P-values of less than 0.05 were considered to be statistically significant.

Results

1. The inhibitory effect of plant extracts on -amylase activity

The results presented in table2 show the inhibitory effect of the aqueous extracts of the five medicinal plants tested in this study. The mixture of graded concentration of extracts with PPA and starch induced a reduction in the enzyme activity and their IC_{50} values calculated demonstrate it. The highest inhibitory activity was observed in the extract PaE, followed by, in a decreasing order: OeE, JoE, OsE and SoEwith IC_{50} equal to 0.61±0.02, 0.99±0.12, 1.20±0.05, 1.23±0.08, 1.30±0.08 mg/ml respectively. The positive control (Acb) has the lower IC_{50} 0.07±0.002mg/ml.

2. The effect of plant extracts on postprandial hyperglycemia

Figure 1-A shows the time course of BGL after oral administration of a single dose (250mg/kg bw) of PaE, OeA, OsE, JoE, SoE or acarbose (50mg/kg bw) with starch (5g/kgbw) to respective group of rats and control (starch+ water).

In the control group, BGL was increased significantly from 0.85 to 1.38g/l (38%) at 30 min and reached 1.39g/l(39%) at 60 min after starch administration, then decline subsequently to basal levels. In experimental and positive control groups, the raise of BGL was attenuated at 30 min except group treated with JoE. They prevent BGL increasing to high level as in the control groups. This antihyperglycemic effect is estimated to be 86% ($p<0.05$) for PaE, 86% ($p<0.05$) for SoE and 61% ($p<0.05$) for Acb. At 60 min, the antihyperglycemic effect was observed in groups treated by PaE, SoE, Acb and OeE at rates estimated to be 74% ($p<0.05$), 51% ($p<0.05$), 41% ($p<0.05$) and 20% respectively.

The effect of plants extracton postprandial hyperglycemia, evaluated by calculation of incremental $AUC_{0\text{-}180min}$, is presented in figure1-B. The oral administration of starch to rats of control group caused an increase of blood glucose concentration within 180 min after starch-loading, $AUC_{0\text{-}180min}$ value reached 60 g/L.min. In treated groups, plants extracts reduced $AUC_{0\text{-}180min}$by 60% for PaE followed by SoE (48%), Acb (34%), OeE (3.89%), OsE (3.21%) and JoE (-8.5%). PaE, SoE and Acb reduced significantly ($p<0.05$) postprandial hyperglycemia in comparison to control group.

Discussion

In this study, we were interested to some medicinal plants used for the treatment of diabetes mellitus by diabetic patients in north western of Algeria. They are screened for their -amylase activity inhibitory potential in vitro and for their effect on postprandial hyperglycemia induced by starch loading test in normal rats.

All plants species tested *in vitro* showed a varying degree of inhibition of amylase activity with IC_{50} value included between 0.61-1.30mg/ml higher than IC_{50} of acarbose which equal to 70µg/ml in line with its known -amylase inhibitory action.

Previous studies related to plant inhibitory potential of -amylase, as study of Bahman et al., 2011 on Iranian medicinal plants report that olive leaf extract show a weak inhibitory effect on -amylase(15.84% inhibition at a concentration of 2.30 mg/ml)[22]. Earlier studies of Komaki et al., 2003, reported that ethanol extract of olive leaves exhibit a high inhibitory effect on human pancreatic amylase (IC_{50} = 0.02mg/ml) compared to hot water extract (IC_{50}=70.2mg/ml). The inhibitory effect against amylase was attributed to luteolin and oleanolic acid [23,24] .

This disseminate results obtained by different authors can be explained, in part, by the influence of agro-ecological conditions on the leaves composition and active compounds content of the olive tree as signaled by Gonzalez et al.1992, previously[24]. Indeed, this author noted that hypoglycemic effect of olive leaf is influenced by season of collect of samples, those collected in the winter months especially in February are the most active.

Concerning *J. oxycedrus*, in our study IC_{50} of roots extract was found to be *1.20*mg/ml. Loizzo et al, 2007, reported that wood essential oil of *J. oxycedrus* exhibited a higher inhibitory effect against amylase with IC_{50} of 3.49µl/ml, while oil obtained from berries exhibited a moderate activity, with IC_{50} value >25µl/ml, which can explain the hypoglycemic activity of this plant[26]. Other studies show also that hydro-alcoholic leaf extract and fruit of *J. oxycedrus* possess moderate inhibitory effect against amylase of 25 to 51.7% and 39-52.6% at a concentration comprised between 0.1-3 mg/ml and1-33 mg/ml respectively [27]. Ultimately, all of these studies support the hypothesis that one of the targets for antidiabetic property of *J. oxycedrus* is pancreatic -amylase inhibition but its effectiveness would depend on the part of plant used and the method of preparation of extract.

In relation to *S. officinalis* and *P. angustifolia,* the most effective plant species against postprandial hyperglycemia, may act by inhibiting the main digestive enzymes: -amylase and -glucosidases (maltase, isomaltase, glucoamylase) responsible for the breakdown of starch and oligosaccharides to glucose as a final product. Their aqueous extract may contain active compounds which inhibit these enzymes. They may also block glucose transport across intestinal mucosa, as possible action and finally reduce its blood level. This hypothesis is comforted by findings of Moradabadi et al, 2013 which showed that *S. officinalis* inhibit strongly maltase activity in experimental rats while itsextract acts

like acarbose [28]. This effect was attributed to rosmarinic acid and other phenolic components of *S. officinalis*[29].

S. officinalis is a well known medicinal and aromatic plant. It was used for many purposes. This plant and other species of genus salvia was intensively studied for their numerous pharmacological properties and active compounds characterization, last years [30]. Itsantidiabetic activity was studied by Eidi al 2005, which reported that *S. officinalis* leaf methanol extract decrease fasting blood glucose level in diabetic rats without effect on insulin releasing from pancreas but not in healthy rats [31]. Lima et al., 2006 reported that sage tea lowers fasting blood glucose in diabetic mice when administrated as a drink. It exhibits a metformin-like effect, inhibiting gluconeogenesis in primary cultures of hepatocytes isolated from lever of diabetic rats[32]. Recently, a clinical study reported that *S officinalis*leaf extract have an antihyperglycemic and lipid profile improvement effects in hyperlipidemic type 2 diabetic patients[33].

P. angustifolia showed the highest -amylase inhibitory activity when compared to the other plant species studied, which can be responsible partially for its effect on reduction of postprandial hyperglycemia. The antihyperglycemic activity *P. angustifolia* has not been reported before and to the best of our knowledge, our results can be assessed as the first report about this property. *P. angustifolia* (Oleaceae) was reported to be used traditionally to treat digestive ailments and infections disease (measles, syphilis) in Eastern Morocco [20], gengitivis and hypertension in Libya [34].This plant lack of scientific evaluation for antidiabetic activity. So it is interesting to be investigated to elucidate its mechanism of action, identify its active compounds implicated in this antihyperglycemic activity, as carbohydrates hydrolyzing enzymes inhibitors and also their possible interference with glucose absorption at small intestinal level.

Conclusion

This study was undertaken to evaluate *in vitro* the inhibitory activity of aqueous extractof five selected plants used in Algeria to treat diabetes, against -amylase a key digestive enzyme related to diabetes mellitus and their effect on postprandial hyperglycemia in normal rats. Our results revealed that among the five plant species, *Salvia officinalis* and *Phillyrea angustifolia* are the most effective on postprandial hyperglycemia comparable to acarbose, a currently use drug. This attribute make them promising candidates as natural source agentsuseful for prevention and treatment of type 2 diabetes. However, pharmacological, phytochemical and toxicological studies are needed especially for *Phillyrea angustifolia* to confirm its efficiency and safety.

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