

EFFECT OF NATURAL PRODUCTS ON SOME GLYCOSIDASES AND THEIR EXPECTED HYPOGLYCEMIC POTENTIAL

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Abstract

Aims: It is to screen many natural product extracts for their *in vitro* and *in vivo* effects on the activities of hepatic α -amylase and α -glucosidase to validate their biological importance.

Study design: Different groups of non-diabetic and diabetic rats were treated by different plants for the *in vivo* study of glycosidases. *In vitro* effect of the plants on the tested enzymes was studied in presence and absence of their aqueous extract.

Place and Duration of Study: Department of Biological & Environmental Sciences, Faculty of Science, Beirut Arab University, Beirut, Lebanon, between March 2013 and February 2014.

Methodology: Enzymes were extracted from the livers of normal rats, also the natural products extracts were prepared for the *in vitro* studies. α -Amylase and α -glucosidase assays were done in the presence and absence of each plant extract. For the *in vivo* studies, normal non-diabetic rats were divided into groups, whereas the first group is a control that includes rats fed on normal food diet. The other groups include rats fed on normal food diet mixed with the tested plant leaves (20 mg/g body weight/ day) Diabetes was induced in diabetic rats by single intraperitoneal injection of streptozotocin. Diabetic rats were divided into groups and treated like the non-diabetic rats.

Results: Only *Thymus vulgaris* and *Origanum vulgare* extracts showed a significant *in vitro* dose-dependent inhibition on α -amylase with IC₅₀ values of 0.2±0.01 and 0.37±0.03 mg/ml, respectively. However, the *in vivo* effect was not detected for four weeks treatment for the two enzymes. The *in vitro* treatment of α -amylase by *Thymus vulgaris* and *Origanum vulgare* extracts exhibited a mixed-type inhibition. Moreover, the *in vivo* inhibition of both extracts on the tested hepatic enzymes was not detected in streptozotocin-induced diabetic rats fed on *Thymus vulgaris* and *Origanum vulgare* for four weeks. Blood sugar level was non-significantly decreased with respect to that of non-treated rats.

Conclusion: some non anti-diabetic plant extracts possess an *in vitro* inhibition of glycosidases.

Key Words: Natural products, α -amylase, α -glucosidase, enzyme inhibitors, diabetes.

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51 1. Introduction

52 Glycosidases (EC 3.2.1.-) are group of enzymes, which were found to play important
53 role in carbohydrate digestion [1]. Thus, the inhibition of their activity may result in
54 lowering and retarding glucose absorption and decreasing blood glucose level (BGL)
55 [2] and is considered as a therapeutic tool [3]. Glycosidases function or dysfunction
56 has been implicated in a number of different disease states, leading to an interest in
57 inhibitors of glycosidases as potential therapeutics [4]. The successful prevention of
58 the onset of diabetes consists in controlling postprandial hyperglycemia by the
59 inhibition of α -glucosidase and α -amylase activities, resulting in aggressive delay of
60 carbohydrate digestion to absorbable monosaccharide [5].

61 On the other hand, plant-based foods have been used in traditional health systems to
62 treat many diseases. Plant extracts have long been used for the treatment of diabetes in
63 various systems of medicine, and are currently accepted as an alternative for diabetic
64 therapy [6]. In addition, their effect on enzyme activity has been widely investigated.
65 Taken together, the control of glycosidases activity and the importance of natural
66 source of glycosidases activators/inhibitors, have a therapeutic potential for their
67 beneficial use in various medicinal systems. Therefore, the present study was
68 undertaken to screen the natural products that can, *in vivo* and/or *in vitro*, inhibit or
69 activate hepatic α -glucosidase and α -amylase in both normal and streptozotocin
70 (STZ)-induced diabetic rats. The present study includes the investigation of the *in*
71 *vitro* and *in vivo* effect of some natural products extracts on hepatic α -glucosidase and
72 α -amylase, followed by kinetic studies of both enzymes with and without *in vitro*
73 treatment. Moreover, the hypoglycemic effect of some extracts on experimentally
74 diabetic rats and their *in vivo* effect on glycosidases will be also investigated.

75

76 2. Materials and Methods

77

78 2.1. Materials

79 The enzymatic colorimetric assay kit for glucose (phenol, 4-amino-antipyrine, glucose
80 oxidase and peroxidase) was purchased from Boehringer, Mannheim, Germany.
81 Bovine serum albumin (BSA), *p*-nitrophenyl- α -D-glucopyranoside, streptozotocin
82 (STZ) and 3,5-dinitrosalicylic acid (DNSA) were purchased from Sigma (Sigma
83 Aldrich, Ibra. Hadad, Beirut, Lebanon). Sephadex G-100 was purchased from

Pharmacia (Uppsala, Sweden). The plants *Thymus vulgaris*, *Origanum vulgare*, *Rosmarinus officinalis*, *Lupinus orientalis*, *Pimpinella schweinfurthii*, *Piper nigrum*, *Camellia sinensis assanica*, *Nigella sativa* and *Camellia sinensis sinensis* were obtained from different areas of the flora of South Lebanon (Table 1).

2.2. Animals

Male albino Sprague- Dawley rats (*Rattus norvegicus*), of body weight, approximately 120 g were obtained from the animal house of the American University of Beirut. Animals were housed in stainless cages (3 - 4 animals/cage) under standardized laboratory conditions with controlled light cycles (12L: 12D), temperature of 23±2°C. Animals had access to tap water and to standard food diet ad libitum. The regional ethical committee (BAU Ethics Committee) approved the animal protocols.

2.3. Methods

2.3.1. Animals treatment

Normal non-diabetic rats were divided into 3 groups (10 rats per each group). Group 1 is a control group that includes rats fed on normal food diet, group 2 includes rats fed on normal food diet mixed with *Thymus vulgaris* (20 mg/g body weight/ day), and group 3 includes rats fed on normal food diet mixed with *Origanum vulgare* (20 mg/g body weight/ day). Diabetes was induced in diabetic rats by single intraperitoneal injection of STZ at a dose of 55 mg/kg body weight [7]. Blood glucose level was measured at days 3, 10, 20 and 30 of STZ injection using One Touch Select glucometer strips. Rats were considered diabetic when blood glucose level is greater than 250 mg/dl [8]. Diabetic rats were divided into 3 groups (7 - 10 rats per each group), whereas group 1 is a control group (control diabetic). It includes rats that received STZ injection and fed on normal food diet. Group 2 includes rats that received STZ injection and fed on normal food diet mixed with 20 mg *Thymus vulgaris* / g body weight/ day, and group 3 includes rats that received STZ injection and fed on normal food diet mixed with 20 mg *Origanum vulgare* / g body weight/ day. After 4 weeks of treatment, the rats were sacrificed, and their livers were taken and stored at -80°C for further studies. A mortality of two rats was recorded during the treatment period.

117 2.3.2. Enzyme purification

118 The purification of hepatic α -amylase from rat liver was performed as previously
119 described [9] according to the known methods [10,11]. Hepatic β -glucuronidase from
120 rat liver was carried out as previously reported [12,13]. All steps were carried out at 0
121 - 4 °C.

122 2.3.3. Enzyme Assays

123 The assay of α -amylase activity was determined as described previously [14]
124 The reaction was carried out by incubating 500 μ l of diluted enzyme and 500 μ l of
125 0.02 mM soluble starch in 0.02 M potassium phosphate buffer, pH 6.9, containing
126 0.0067 M NaCl at 37°C for 30 min. The reaction was interrupted by the addition of
127 3,5-dinitrosalicylic acid reagent. Finally, the mixture was heated for 5 min in a boiling
128 water bath and cooled under running tap water. After the addition of 10 ml water, the
129 brown color was measured at 540 nm. The concentration of liberated maltose was
130 determined using maltose standard curve [13]. One unit of enzyme activity hydrolyzes
131 1 mmol of the disaccharide per min at 37 °C. The assay of α -glucosidase was carried
132 out by incubating 50 μ l of enzyme with 450 μ l of 20 mM *p*-nitrophenyl- α -D-
133 glucopyranoside in 50 mM potassium phosphate buffer, pH 6.9 at 37°C for 30 min.
134 The reaction was stopped by adding 0.5 ml of 0.2 M carbonate-bicarbonate buffer, pH
135 10.4. The obtained yellow color formed by liberated *p*-nitrophenol under alkaline
136 condition, is measured at 410 nm. The concentration of liberated *p*-nitrophenol was
137 determined using *p*-nitrophenol standard curve [1]. One unit of α -glucosidase activity
138 is defined as the amount of enzyme, which hydrolyzes 1 m mole of *p*-nitrophenyl α -D-
139 Glucopyranoside per minute at 37°C. Specific activity is expressed as units per mg
140 enzyme protein. All assays were done in triplicates and the results were represented as
141 mean $\pm$ S.E.

143 2.3.4. Protein Assay

144 The protein content of the crude enzyme source was determined by using bovine
145 serum albumin as a standard [15].

147 2.3.5. Natural products extracts

148 The aqueous extracts were obtained from *Thymus vulgaris*, *Origanum vulgare*,
149 *Rosmarinus officinalis*, *Lupinus orientalis*, *Pimpinella schweinfurthii*, *Piper nigrum*,

Camellia sinensis assanic, *Nigella sativa* and *Camellia sinensis sinensis*. The subjected plants were homogenized in 10 mM potassium phosphate buffer, pH 7.2 containing 1 mM EDTA and 0.25 M sucrose (7 volumes of plant weight), using mortar and pestle (seeds were kept in buffer solution for 2 nights before being homogenized). The obtained homogenate were passed through muslin first, and then centrifuged at 3000 rpm for 30 min at 4°C.

2.3.6. Kinetic studies of in vitro treated enzyme

Time course of the partially purified enzymes were done at different substrate concentrations, in the presence and absence of 3 doses of *Thymus vulgaris* and *Origanum vulgare* extracts. In each time course, initial velocity value (v_0) was calculated. These values were used to plot Dixon and Line-weaver Burk plots that were used to determine the type of inhibition.

2.3.7. Statistical analysis

The values were statistically analyzed using student t-test and paired sample t-test (SPSS version 16). Data were expressed as mean $\pm$ SE. Values with $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. In vitro screening of natural products for hepatic enzymes

In order to screen for natural products that have an effect on hepatic α -amylase activity, the assay of the enzyme in the presence and absence of different doses of each plant extracts was done. In parallel to these assays, a control assay was performed (without any extract). The comparison between the specific activities of the control and other assays provides us an idea about the effect of each extract on α -amylase activity. The change of the relative specific activity of hepatic α -amylase (percentage of control) with the concentration of four natural products extracts was shown in Fig. 1 A. The results showed that only *Thymus vulgaris* and *Origanum vulgare* and extracts exerted a significant dose-dependent inhibition on α -amylase specific activity. *Origanum vulgare* exerted a significant inhibition starting from 0.3 mg / ml dose, which then became highly significant starting from 0.6 mg / ml dose. *Thymus vulgaris* showed a significant inhibition starting from 0.1 mg/ml dose but did not show highly significant effect at the doses used in the experiment. The observed

184 IC50 were found to be 0.2 ± 0.01 and 0.37 ± 0.03 mg/ml for *Thymus vulgaris* and
185 *Origanum vulgare*, respectively. Other natural products also showed a dose-dependent
186 inhibition but the effect was not significant, whereas the remaining activities at the
187 higher concentration ranged 81.5, 88.8, 90.3, 90.5, 97.8 and 99.28% for *Lupinus*
188 *orientalis*, *Rosmarinus officinalis*, *Pimpinella schweinfurthii*, *Piper nigrum*, *Camellia*
189 *sinensis assanic*, *Nigella sativa* and *Camellia sinensis sinensis*, respectively (Figs. 1 A
190 & B). Fig. 2 illustrates the relative specific activity of the positive control
191 diethanolamine (DEA) as a ratio of the value in presence to that of control, whereas
192 the specific activity of α -amylase is expressed as a relative value compared to control.

193

194 The effects of the same nine extracts have been also studied on hepatic α -glucosidase
195 activity. The specific activity of hepatic α -glucosidase in the absence (control) and
196 presence of different doses of nine natural products extracts. The results showed that
197 none of the used extracts exerted a significant effect on α -glucosidase specific
198 activity. A non-significant dose-dependent inhibition on α -glucosidase was recorded
199 as 8.0 and 2.0 % decrease of the relative specific activity was recorded for both
200 *Thymus vulgaris* and *Origanum vulgare*, respectively. The effect of the other extracts
201 was negligible.

202

203 **3.2. Kinetic studies of *in vitro*-treated α -amylase by *Thymus vulgaris* and** 204 ***Origanum vulgare* extracts**

205 The study of the type of *in vitro* inhibition of α -amylase by *Thymus vulgaris* and
206 *Origanum vulgare* extracts involves measurement of the kinetic parameters of the
207 enzyme. The time course of the enzyme reaction was done at different substrate
208 concentrations in the absence for the determination of the initial velocity values (v_0)
209 of the reactions. Panels A and B of Fig. 3 represent Lineweaver-Burk and Dixon plots,
210 respectively for hepatic α -amylase with different doses of *Thymus vulgaris*.
211 Lineweaver-Burk plot shows straight lines that intersect at the left side of the y-axis,
212 and Dixon plot shows straight lines that intersect at the y-axis. Both results are
213 characteristics of mixed type inhibition. The same results were obtained for
214 *Origanum vulgare* extracts in panels C and D of Fig. 3.

215

216 **3.3. Measurement of BGL in STZ - induced diabetic rats**

Blood glucose level (BGL) was measured in the STZ-induced diabetic rats pre and post STZ injection. BGL was first measured at day zero using the glucometer, then the rats received single intraperitoneal injection of STZ and its BGL was measured at day 3, 10, 20 and 30 post STZ injection. Diabetic rats were divided randomly into 3 groups before STZ injection. All rats received the same STZ dose. In all groups BGL measured at day zero, which served as control, was almost the same around 100 mg/dl. The value then increased significantly in the groups compared to the control ($p \leq 0.05$) starting from 3rd day-post STZ injection (Fig. 4). The treated groups were fed daily on *Thymus vulgaris* and *Origanum vulgare* extracts (20 mg/g body weight/day), that were mixed with the food of the rats for 30 days. BGL as mg/dl in the treated groups with *Thymus vulgaris* and *Origanum vulgare* (304 ± 25 and 291 ± 29 respectively) recorded lower values than that of non-treated group (diabetic control) (350 ± 35). This difference was observed from 3rd day-post STZ injection until the end of the treatment (day 30).

3.4. *In vivo* effect of *Thymus vulgaris* and *Origanum vulgare* on enzymes

In order to study the *in vivo* effect of *Thymus vulgaris* and *Origanum vulgare* on hepatic α -amylase in both normal non-diabetic and STZ-induced diabetic rats, all rat groups were sacrificed at day 30 of treatment and their livers were taken in order to perform the assay of α -amylase. The relative specific activity value obtained from untreated rats was considered as controls (100 %) in normal and diabetic rats (control normal and control diabetic). Fig. 5 shows the results of α -amylase assay in all rat groups. These results indicate that the two plants did not exert an *in vivo* effect on hepatic α -amylase. The relative enzyme specific activity showed a significant increase in diabetic versus non-diabetic rats. On the other hand, the plant extract did not exert any *in vivo* effect on hepatic α -glucosidase and the relative enzyme specific activity in diabetic rats show a slightly less than the activity in non-diabetic ones as non-significant value.

4. Discussion

Starch digestion in the human body is typically viewed in a sequential manner beginning with α -amylase followed by α -glucosidase to produce glucose [16]. Therefore, a current therapeutic strategy for the control of postprandial hyperglycemia is the inhibition of α -glucosidase and α -amylase, resulting in aggressive delay of

carbohydrate digestion to absorbable monosaccharide [5]. This is the main reason of using these enzymes in the current study. STZ was used to induce diabetes and animals with blood glucose level above 250 mg / dl are considered diabetic [8,17].

In the present study, the obtained results showed a significant increase in BGL at day 3 post - STZ injection in all STZ – injected groups and this is consistent with what had been obtained previously [8,17]. Moreover, in the groups fed on *Thymus vulgaris* and *Origanum vulgare*, the increase in BGL was also significant at day 3 post STZ injection. However, the results showed that BGL in the treated diabetic rats is non-significantly decreases compared to the control diabetic group. This non-significant decrease may be due to the treatment duration, which was not enough to show a significant change. These results suggest that the plants may act on mechanisms that lead to decrease in BGL, other than α -amylase and α -glucosidase inhibition, because the inhibition of these enzymes by *Thymus vulgaris* and *Origanum vulgare* was observed only in the *in vitro* and not in the *in vivo* experiments. These mechanisms may include other enzymes that are also involved in carbohydrate digestion, or it could be inhibition of the same enzymes (α -amylase and α -glucosidase) but not from hepatic source, such as pancreatic and intestinal enzymes. Also, these plants may have an effect on glucose absorption from the gut and may prolong the absorption process, suppressing the peak blood glucose levels [6]. The confirmation of these results requires other studies that involve longer treatment duration and other enzymes or other sources of these glycosidases (pancreatic or intestinal).

Diabetes mellitus is a metabolic disorder associated with changes in the enzymatic activity of several enzymes. Some enzymes are found to have increased activities, others have decreased activities and others retain the same activity [18]. Glycosidases are subject to changes in their activities in case of diabetes. In the present study, the activity of α -amylase increased significantly in diabetic rats with respect to non-diabetic ones, in both control and treated groups. These results were also observed in other studies that also showed an increased α -amylase activity in diabetes [19,20]. The expected reason of this increase, according to Mendez *et al* could be that in patients with diabetes mellitus, the release of α -amylase into the blood is decreased so its amount and activity in tissues is increased. The increase in α -amylase activity in case of diabetes may contribute to the increased in circulating blood glucose, due to the

high amount of glucose released by α -amylase catalyzed reactions [20]. On the other hand, α -glucosidase activity in the present study slightly decreases in diabetic with respect to non-diabetic rats, which confirms the results in other studies that also show the same change in this enzyme activity [21]. However, the real reason behind this change is not well recognized yet. It could be a kind of negative feedback exerted by the high amount of glucose in blood, which is the major product of α -glucosidase catalyzed reactions.

Not only synthetic compounds, but also natural resources provide a huge and highly diversified chemical bank from which we can explore potential therapeutic agents [22]. Opportunity of exploring medicinal plants is still very wide open in line with the development of herbal industry, herbal medicine, and phytopharmaca. Therefore, researchers are trying to explore the potential antidiabetic agents with the mechanism of action of glycosidases inhibition in several plant species [23]. In fact, it was found that *Nigella Sativa* and *Camellia Sinensis* were proved to have anti-obesity action [24]. *Rosmarinus officinalis* is used to relief respiratory disorders and stimulate growth of hair, its extracts relaxes smooth muscles of trachea and intestine, and has choleric, hepatoprotective and antitumorigenic activity [25]. *Origanum vulgare* and *Piper nigrum* are natural sources of antioxidants [26,27]. *Lupinus orientalis* and *Thymus vulgaris* was tested for its anti-microbial action [28] and effect on inflammatory response respectively [29].

However, it was reported that screened α -glucosidase inhibitors for forty-five plants extracts reveal that the inhibitory activity of this enzyme may be due to the presence of glycoside content in the studied extracts. Glycosides consist of sugars that may be structurally similar to carbohydrate, which is a substrate of the enzyme α -amylase [30]. This suggestion was also mentioned in other studies that studied glycosidases inhibition using natural products [23]. Therefore, this may be the same reason behind the observed inhibition exerted by *Thymus vulgaris* and *Origanum vulgare* extracts on hepatic α -amylase. Moreover, compounds with glycosidases inhibitory activity were preliminary identified by the existence of alkaloid, flavonoid, phenolics and other compounds in their structure [23]. Some studies mentioned that *Thymus vulgaris* and *Origanum vulgare* contain flavonoid and phenolic compounds [31]. Therefore, the inhibition of the tested enzymes in the current study may be attributed to these

compounds. The obtained inhibition by both extracts occurred in a dose-dependent manner. The concentration-dependent manner of the *in vitro* inhibition indicated that a rapid interaction between these inhibitors and tested enzymes might have taken place [13]. The inhibitory *in vitro* effect of *Thymus vulgaris* and *Origanum vulgare* on hepatic α -amylase was not observed on hepatic α -glucosidase. This may be due to the difference in the structure of the active sites, so that the compounds that block or interrupt the action of α -amylase do not match or affect α -glucosidase active site. However, not always does the *in vitro* inhibitory activity relate to the corresponding *in vivo* activity [6].

Thus, proof of concept needed to be demonstrated in animal studies. For safety and efficacy to be established, it was essential to confirm the *in vivo* action following oral administration to different rats groups. Hence the *in vivo* experiments were performed. The obtained results showed that the observed *in vitro* effect on hepatic α -amylase was not observed *in vivo* on hepatic enzyme of rats fed on *Thymus vulgaris* and *Origanum vulgare*. This may be due to the digestion and metabolism of the plants components that lead to the breaking down of effective inhibitory compound present in these plants, so their effect on hepatic α -amylase was lost. This difference between *in vitro* and *in vivo* effects was also observed in many drugs whose activity is decreased or even lost *in vivo* treatment [32].

In the present study, the extracts of both *Origanum vulgare* and *Thymus vulgaris* displayed a mixed- type inhibition on α -amylase. On the other hand, both extracts did not exert an effect on α -glucosidase activity. However, the inhibition of glycosidases can have profound effects on quality control, maturation, transport, and secretion of glycoproteins and can alter cell-cell or cell-virus recognition processes. This principle is the basis of the potential use of glycosidase inhibitors for viral infection, cancer, and genetic disorders. Glycosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases [33,34]. However, the hepatic enzyme in the liver has a high homology to the pancreatic enzymes in its primary structure [35]. Therefore, the hepatic enzyme but not the pancreatic one was applied in the present study to get a considerable purified amount.

353

354 In future, specific inhibitor has to be isolated from the crude extract, characterized and
355 therapeutically exploited. Therefore, current attempts to purify the active compounds
356 from extracts should be conducted to understand the inhibitory mechanisms more
357 clearly, also studies with other enzymes and inhibition analyses are needed to
358 determine the reason behind BGL decrease after feeding on *Thymus vulgaris* and
359 *Origanum vulgare*. These results further support the traditional use of plants in
360 medicine based on their inhibitory activity on carbohydrate digestion and glucose
361 absorption. Although these extract seems to be promising target in reducing
362 postprandial hyperglycemia, it is still early to recommend its frequent use in humans
363 due to the requirement of further studies.

364

365 In conclusion, *Thymus vulgaris* and *Origanum vulgare* extracts showed a significant
366 *in vitro* inhibitory effect on hepatic α -amylase with a mixed-type mode of inhibition.
367 This effect was not observed *in vitro* on hepatic α -glucosidase and *in vivo* on both
368 enzymes. These plants have a non-significant decrease in BGL in treated diabetic rats
369 more than the non-treated diabetic group (diabetic control).

370

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376

377 Conflict of interest

378 The authors report no conflict of interest.

379

380 Figure legends:

381

382 **Fig. 1. *In vitro* effect of different doses of the extract of *Thymus vulgaris*,
383 *Origanum vulgare*, *Rosmarinus officinalis* and *Lupinus orientalis* (A) or *Pimpinella*
384 *schweinfurthii*, *Piper nigrum*, *Camellia sinensis assanica*, *Nigella sativa* and
385 *Camellia sinensis sinensis* (B) on hepatic α -amylase.** Specific activity of α -amylase
386 is expressed as a relative percentage. Natural products doses are expressed as mg/ml.

387 **Fig. 2. Dose-dependent effect of the positive control DEA.** Specific activity of α -
388 amylase is expressed as a relative value compared to 1.0 as control. Doses of DEA are
389 expressed in μ g per reaction mixture.

390 **Fig. 3. Kinetic studies of hepatic α -amylase. The reciprocal plot of the enzyme**
391 **was studied at different concentrations of *Thymus vulgaris* (A) or *Origanum***
392 ***vulgare* (C) extracts. Dixon plot of the enzyme was studied at different**
393 **concentrations of *Thymus vulgaris* (B) or *Origanum vulgare* (D) extracts.** Starch
394 concentration is expressed in μM and v_o is expressed in $\mu\text{M}/\text{min}$. The concentration of
395 natural products is expressed in mg/ml and v_o is expressed in $\mu\text{M}/\text{min}$.

396 **Fig. 4. Time evolution of BGL post intraperitoneal injection of STZ. * $p \leq 0.05$ vs**
397 **day zero.**

398 **Fig. 5. *In vivo* effect of *Origanum vulgare* and *Thymus vulgaris* on hepatic α -**
399 **amylase and α -Glucosidase in diabetic and non-diabetic rats. Rats received 20**
400 **mg/g body weight for four weeks. Specific activity of α -amylase is expressed as a**
401 **relative percentage.. Significant, * $p \leq 0.05$ vs non-diabetic.**

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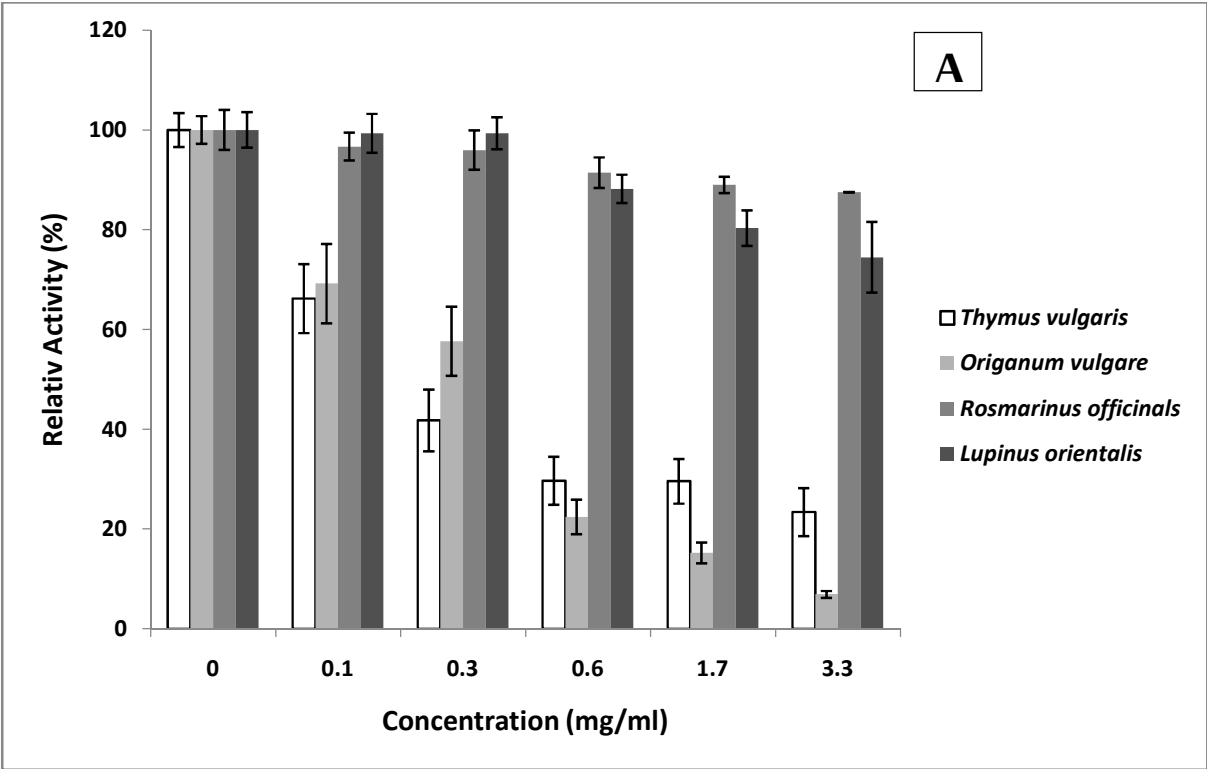
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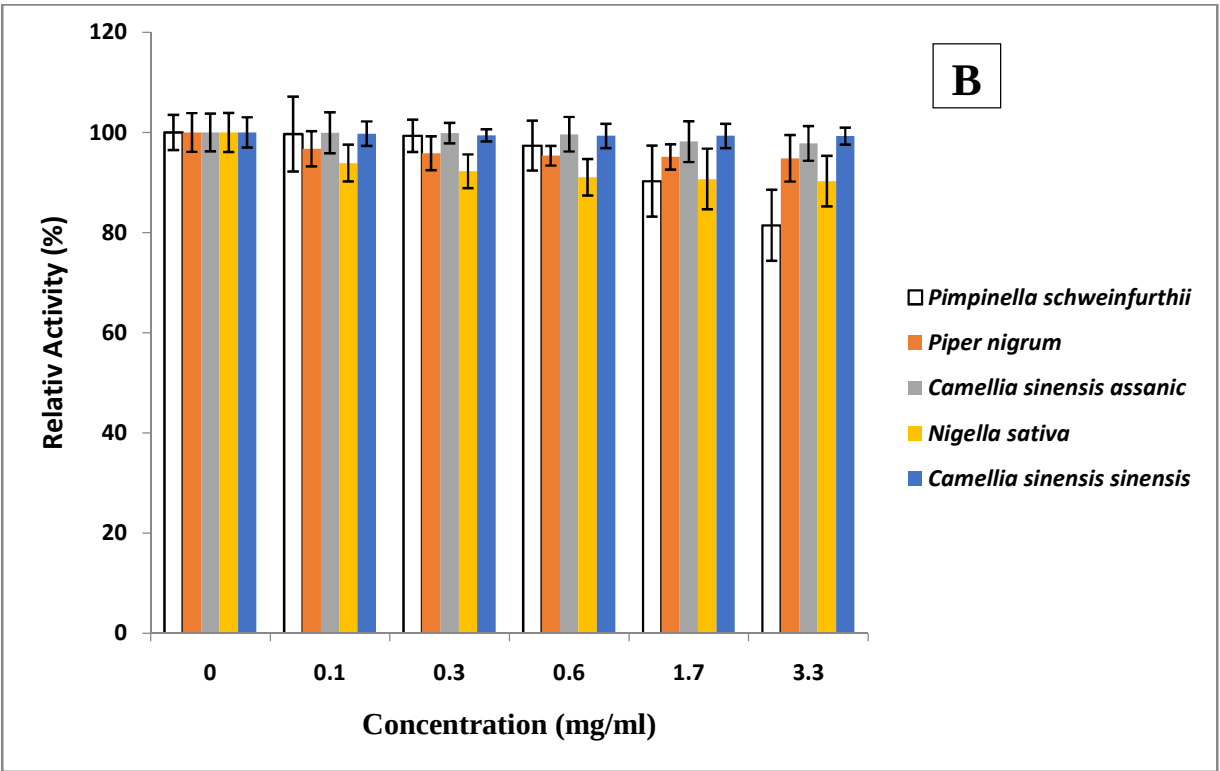
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512 **Fig. 1**

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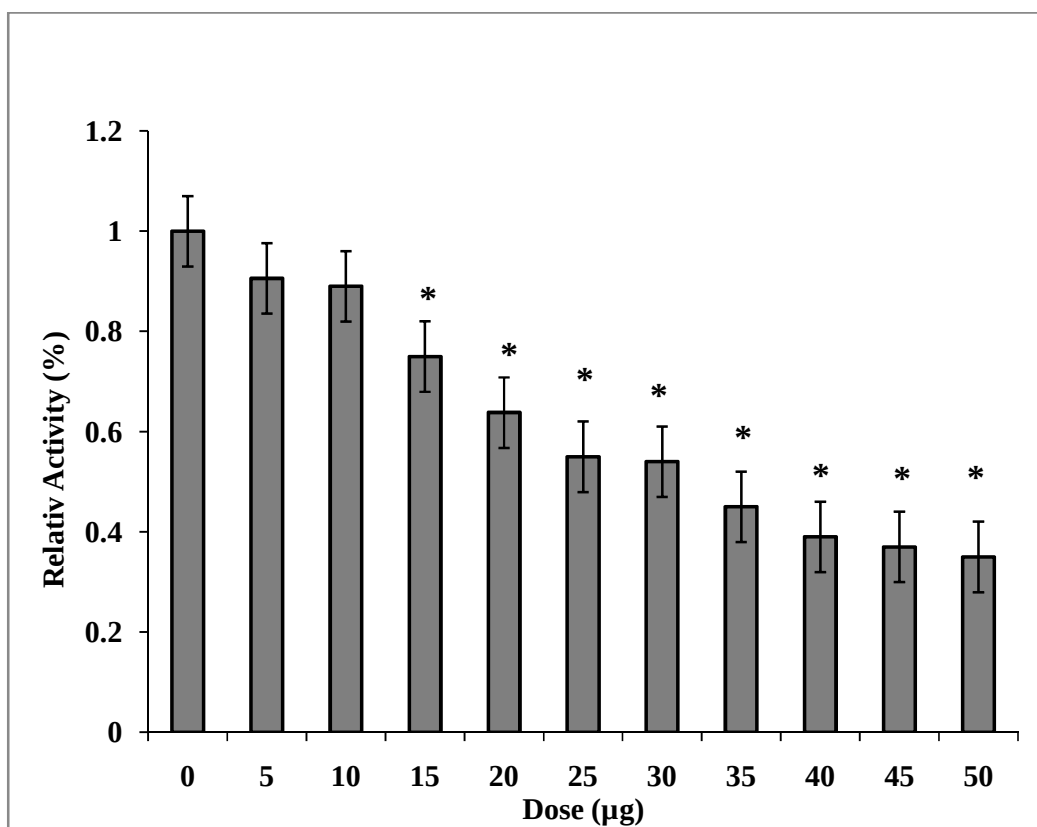


Fig. 2

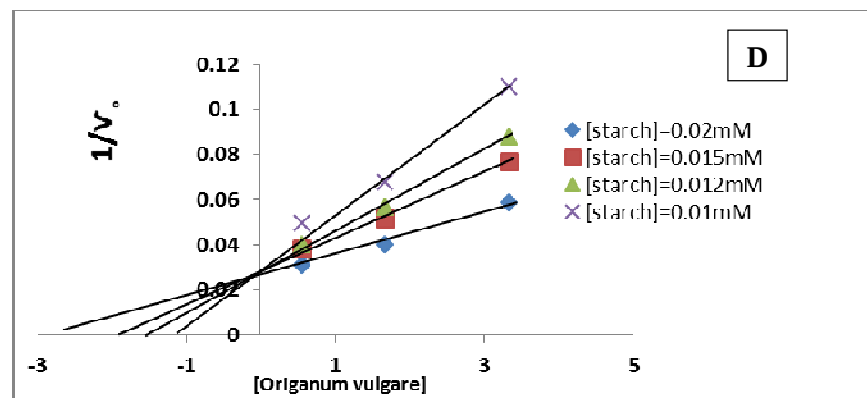
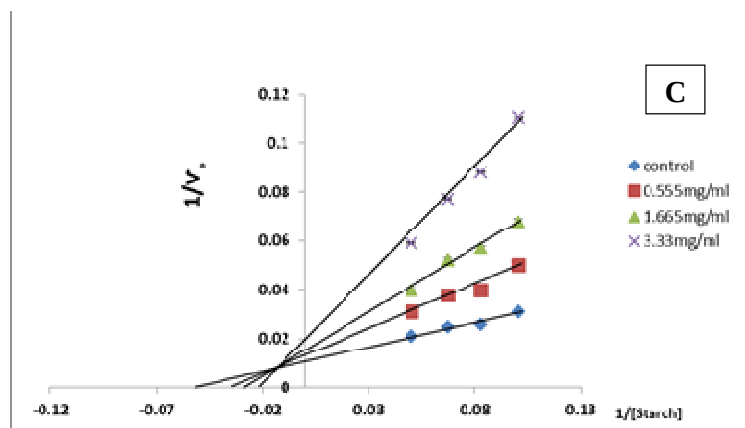
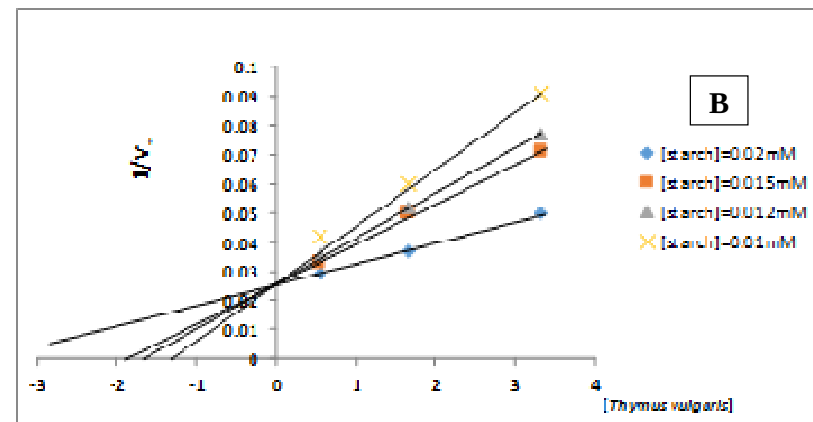
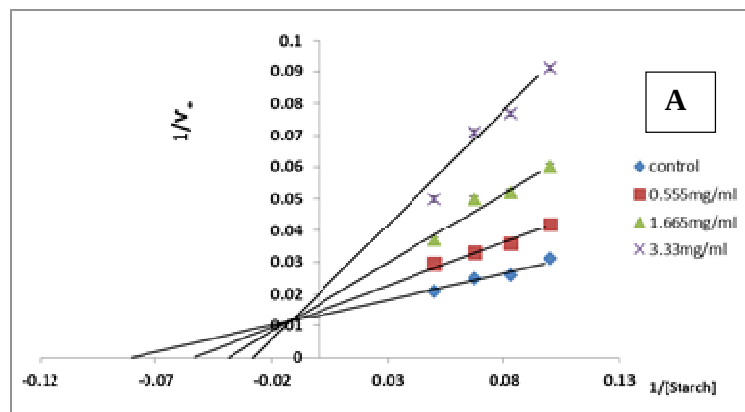


Fig. 3

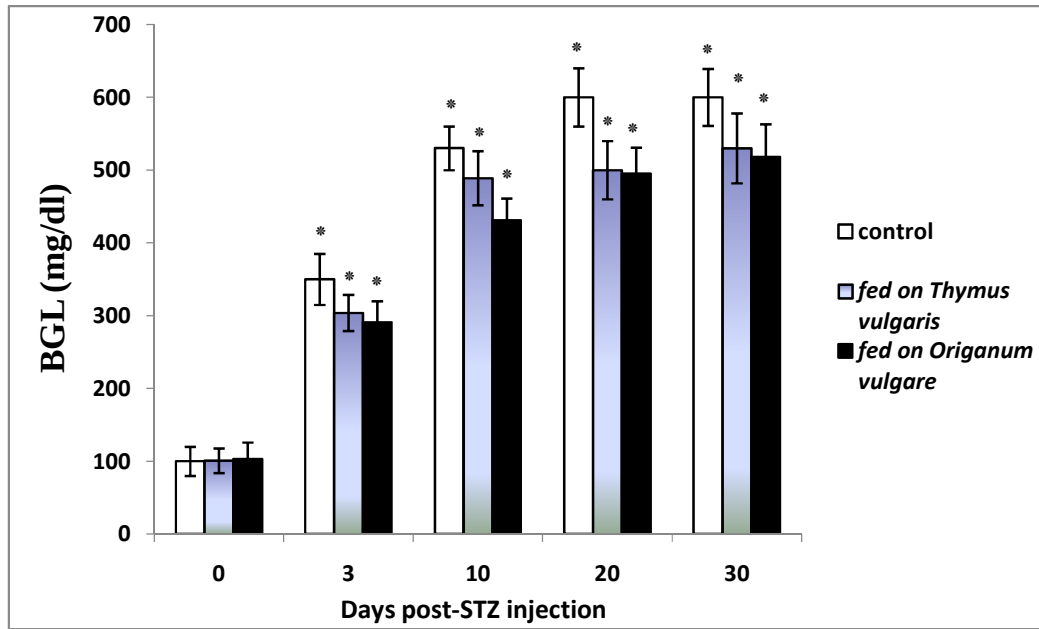


Fig. 4

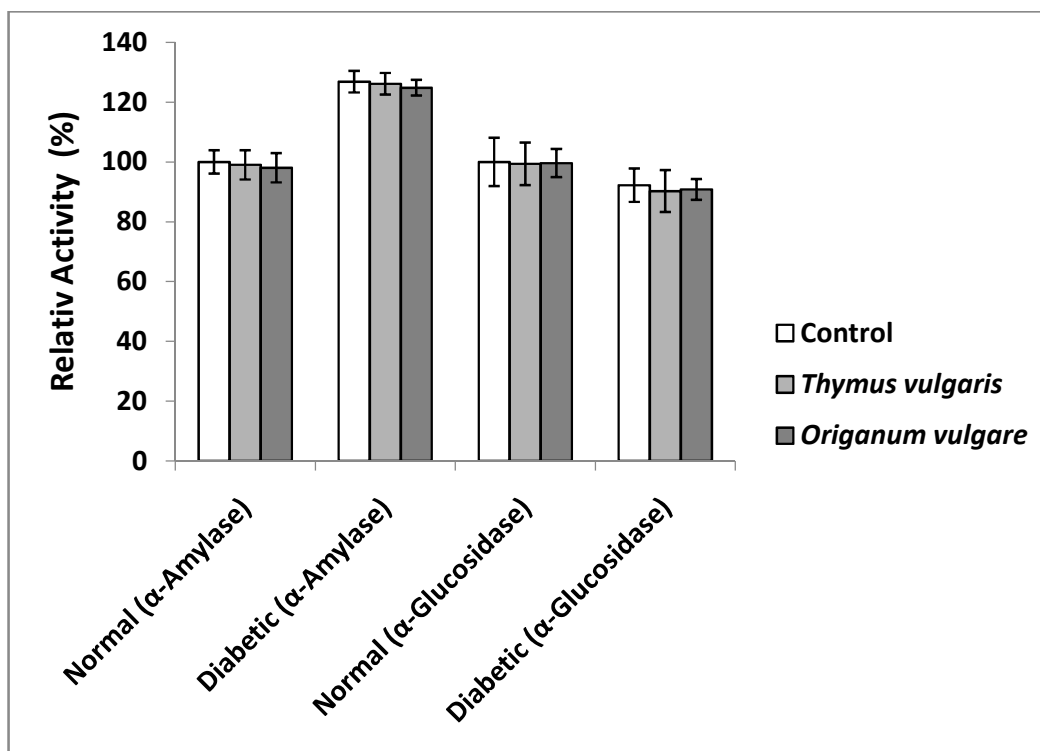


Fig. 5

Table 1. Voucher number, common name, family, time of collection, place of collection and parts used of the selected Lebanese medicinal plants.

Plant	Voucher Number	Common Name	Family	Time of Collection	Place of Collection	Parts Used
<i>Thymus vulgaris</i>	Flora of Lebanon	Thyme	Laminaceae	April 2013	South Lebanon	Leaves
<i>Origanum vulgare</i>		Oregano	Laminaceae	April 2013	South Lebanon	Leaves
<i>Rosmarinus officinalis</i>		Common rosemary	Laminaceae	May 2013	South Lebanon	Leaves
<i>Lupinus orientalis</i>		Lupine	Fabaceae	May 2013	South Lebanon	Seeds
<i>Pimpinella schweinfurthii</i>		Aniseed	Apiaceae	April 2013	South Lebanon	Seeds
<i>Piper nigrum</i>		Black Pepper	Piperaceae	May 2013	South Lebanon	Seeds
<i>Camellia sinensis assanic</i>		Black Tea	Brassicaceae	April 2013	South Lebanon	Leaves
<i>Nigella sativa</i>		Black cumin	Ranunculaceae	May 2013	South Lebanon	Seeds
<i>Camellia sinensis sinensis</i>		Green Tea	Brassicaceae	May 2013	South Lebanon	Leaves