

## Inhibitory effect of gundelia extract on urinary $\alpha$ -amylase activity of type-i diabetes mellitus

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### ABSTRACT

**Background and Objectives:**  $\alpha$ -amylase is an enzyme that degrades starch into maltose and glucose by hydrolyzing  $\alpha$ -1,4-glucan bonds. It is known that the enzyme is found to be elevated in patients suffering from diabetes mellitus. A potent extract of locally distributed wild plant, the Gundelia was found to inhibit elevated activity of  $\alpha$ -amylase with dose responses.

**Methods:** Samples of 50 diabetes mellitus type-I has been investigated for urinary  $\alpha$ -amylase activity. An inhibitory dose of Gundelia extract were used for the inhibition of the elevated enzyme activity. The data obtained were compared with that of healthy controlled samples (30). The adopted protocol was a colorimetric determination (Anonymous, 1980).

**Results:**  $\alpha$ -amylase activity was found to be elevated ( $110.5 \pm 10.2$  IU/24hr) in patient's serum compared with that of controlled samples ( $43.28 \pm 3.23$  IU/24hr). The activity of the enzyme was found to be inhibited ( $16.11 \pm 20.4$  IU/hr) in patients serum using dose response of 15mg/ml of Gundelia extract. The study were comprehensive to determined the physical parameters of the enzyme activity and a values of  $V_{max}$  and  $K_m$  were obtained.

**Conclusions:** It was known that Gundelia is used in prevention and treatment of liver diseases. The plant has a role in the body as an antioxidant factor. It has a hypolipemic effect, therefore, a use of such plant extract could have a hypoglycemic activities on patients with DM-I.

**Keyword:**  $\alpha$ -Amylase, Gundelia extract, Diabetes mellitus-I-.

### INTRODUCTION:

Enzyme represents major components of the biological fluids. They are maintained life continuity throughout their catalysis reactions.  $\alpha$ - amylase ( $\alpha$ - 1-4-glucan 4-glucohydrolase, EC 3.2.1.1) is an enzyme that degrades starch, first to oligosaccharides and then in turn to maltose and glucose, by hydrolyzing  $\alpha$ - 1, 4 - glucan bonds. In digestion, the role of  $\alpha$ -amylase is primarily the first reaction of this process, generating oligosaccharides that are hydrolyzed by other enzymes<sup>1</sup>. The enzyme is found in saliva and pancreatic secretions, where it serves an obvious role in polysaccharide digestion. More surprisingly,  $\alpha$ - amylase is also found in

and tears, possibly for anti-bacterial activity<sup>2</sup>.  $\alpha$ - amylase determination has been recognized as an important diagnostic tool for many years<sup>3-5</sup>, because elevated levels of the enzyme are associated with liver and pancreatic disorders, as well as other disease. Increased amylase and lipase occurs 16-25% of the time in diabetes ketoacidosis DKA<sup>6</sup>. Acute pancreatitis can present or coexist with DKA have been reported<sup>7</sup>. Significant, but nonspecific, elevation of amylase can be seen in DKA<sup>8</sup>. Modern researches have shown that the action of medicinal plants is due to a relatively small number of constituents called the active principles by the plant<sup>9</sup>.

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**Gundelia tournefortii L.:**

The gundelia is a spiny, thistle-like flowering plant of the genus *Gundelia* L. in the sunflower family (Asteraceae). They occur in the semi-desert areas of Armenia, Asia Minor, Iraq and Iran<sup>10</sup>. It has been known that gundelia extracts even solids or oils have an extreme effect on enzyme constituents of living cells, therefore, the aim of this study was focusing on the effect of gundelia solid extract on  $\alpha$ -amylase activity.

**MATERIALS AND METHODS:****Extraction of plant**

The leaves of *Gundelia tournefortii* L. have been collected in July 2006 in Sulaimania city (Kurdistan region). The plants were dried for 3–4 weeks in room temperature. They were later grounded through a willy mill and oils were extracted with petroleum ether (40–60°C), using Soxhlet apparatus according to AAcc methods (11), the extracted plant then dried and used in this procedure.

**Chemical:**

All chemicals used in this project were of high analytical grade. Amylase – Kit from BIOLABO REAGENTS was used for Amylase activity.

**Sampling:**

Diabetes Mellitus (I) patients (50 samples) of child ages (1–16 years). The samples were diagnosed by consultants and proved by D.M tests. There are no accompanied diseases. Healthy normal (30 samples) were used as controls, they were of child age (1–16 years).

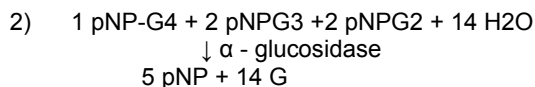
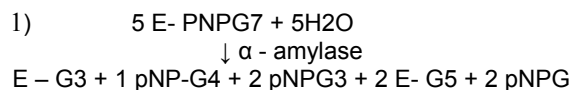
**Collection of Urine:**

Urine (5ml) was collected from patients and healthy control objects. The urine left at room temperature for (1min), and then centrifuged at 3000 rpm for 10 min; urine obtained was used on the same day of experiment.

**Determination of urine – amylase activity:**

Several procedures are available to assay  $\alpha$ -amylase activity (Amyloclastic methods. Saccharogenic methods). These methods

include linearity, sensitivity and precision when compared to E – PNPG 7 method (11), the method adopted in this paper. Reaction scheme is as follows:



E = Ethyliden, PNP= paranitrophenol, G= glucose

The rate of formation of pNP (p-Nitrophenol), directly proportional to the  $\alpha$ -amylase activity in the specimen, is measured at 405 nm.

**RESULT:**

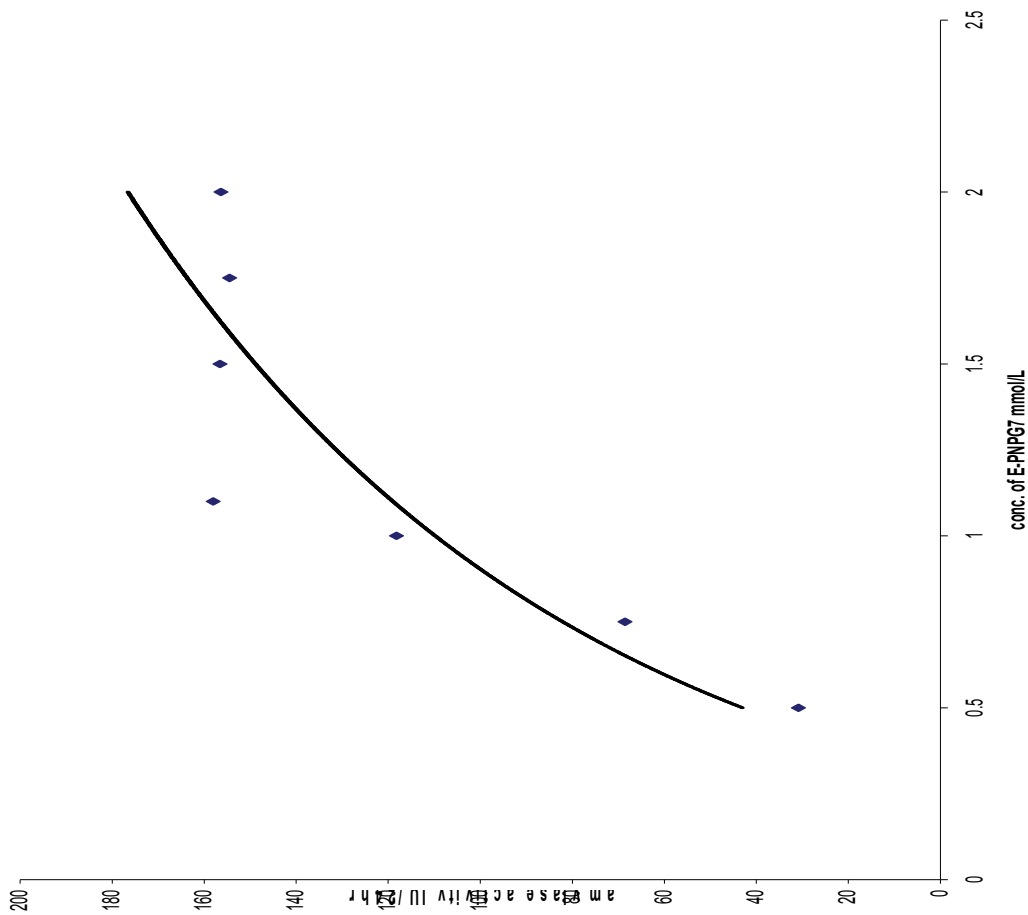
The effect of substrate concentration (0.5, 0.75, 1, 1.1, 1.5, 1.75, 2 mmol/L) on the enzyme analysis was determined and  $K_m$  of 1.1 mmol/L for E-PNPG7 as substrate was found,  $V_{max}$  and  $K_m$  values determined from Michaelis – Menten analysis. These values were confirmed using Lineweaver- Burk plot analysis. The determined data include urine of D.M I. and compared with that of controls. Investigating urine amylase activity, data obtained shows an inhibition of amylase activity in D.M I. (160.11 – 250.41 IU/24 hr) when compared with normal activity (23.98 – 65.39 IU/24hr) tab(1). Determination of  $V_{max}$  values, data obtained from E-PNPG7 [substrate] without inhibitor show 172.41 with  $K_m$  value (0.5) where  $V_{max}$  for [substrate + inhibitor] have same  $V_{max}$  but higher  $K_m$  value (0.8) tab.2. These values of  $V_{max}$  and  $K_m$  were achieved using Michaelis – Menten analysis or substrate (fig-1) and for [substrate + inhibitor] (fig-2). The data analyzed were confirmed by Lineweaver – Burk plot [S] fig-3, and for [S+I] fig-4. Studying the effect of the inhibitor concentration on the rate reaction.

**Table 1:** Amylase activity in the urine of normal control and Patients with D.M.I

Disease	Mean ± SD IU/24 hr	Mean±SD IU/24 hr
Normal N=30	43.38 ± 3.33	23.98 ± 65.39
Diabetes Mellitus(I) N=50	281.70 ± 10.03	160.11 ± 250.4

**Table 2:** Values of Vmax , Km for normal and inhibited [S+I] activity

Substrate	Vmax	Km
E-PNPG7	172.413	0.5
E-PNPG7 + I	172.413	0.8



fig(1)-Michaelis-Menten hyperbolic plot of amylase activity Vs different conc. of E-PNPG7 mmol/L

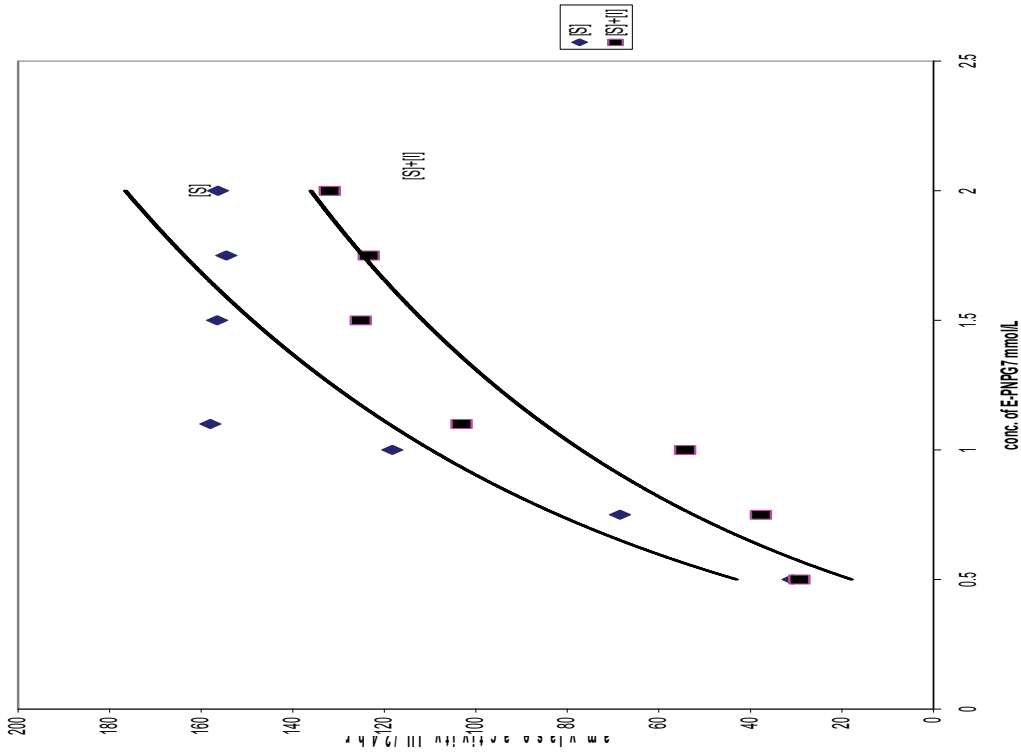
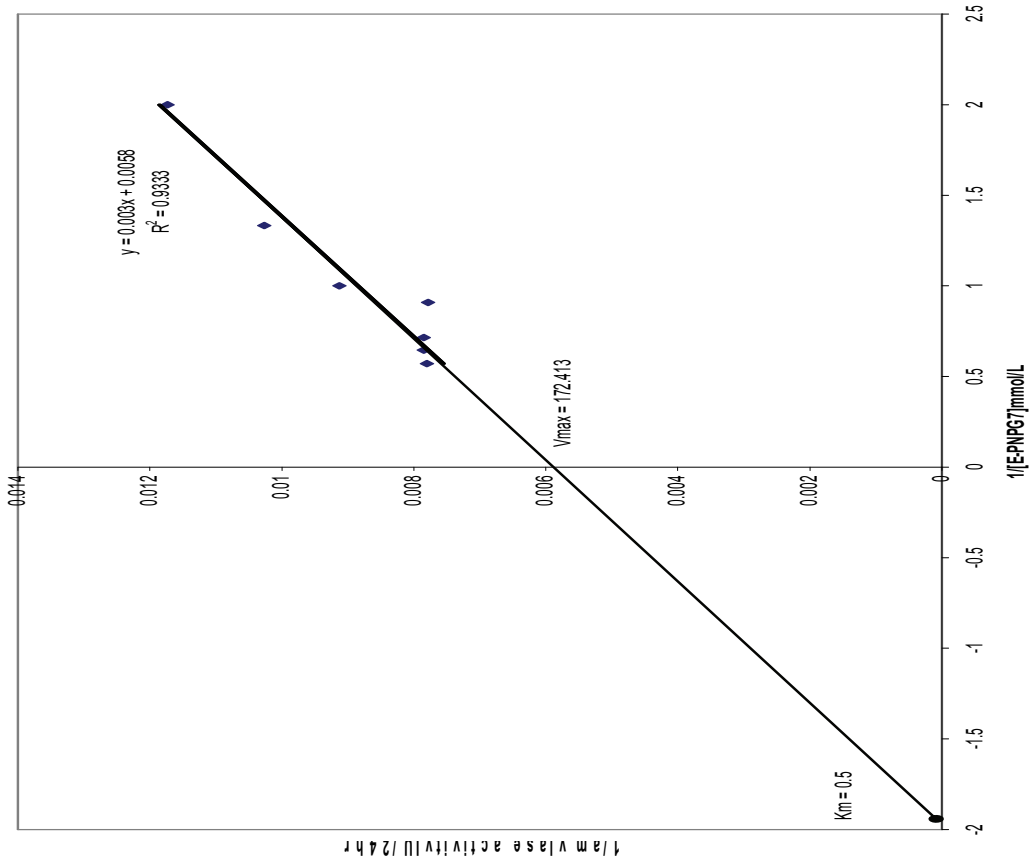


Fig (2)-inhibitory effect of constant dose (15 mg /ml) of extract (A1)

fig-3-Lineweaver-burk plot of amylase activity for different conc. of E-PNPG7 /mmol/L



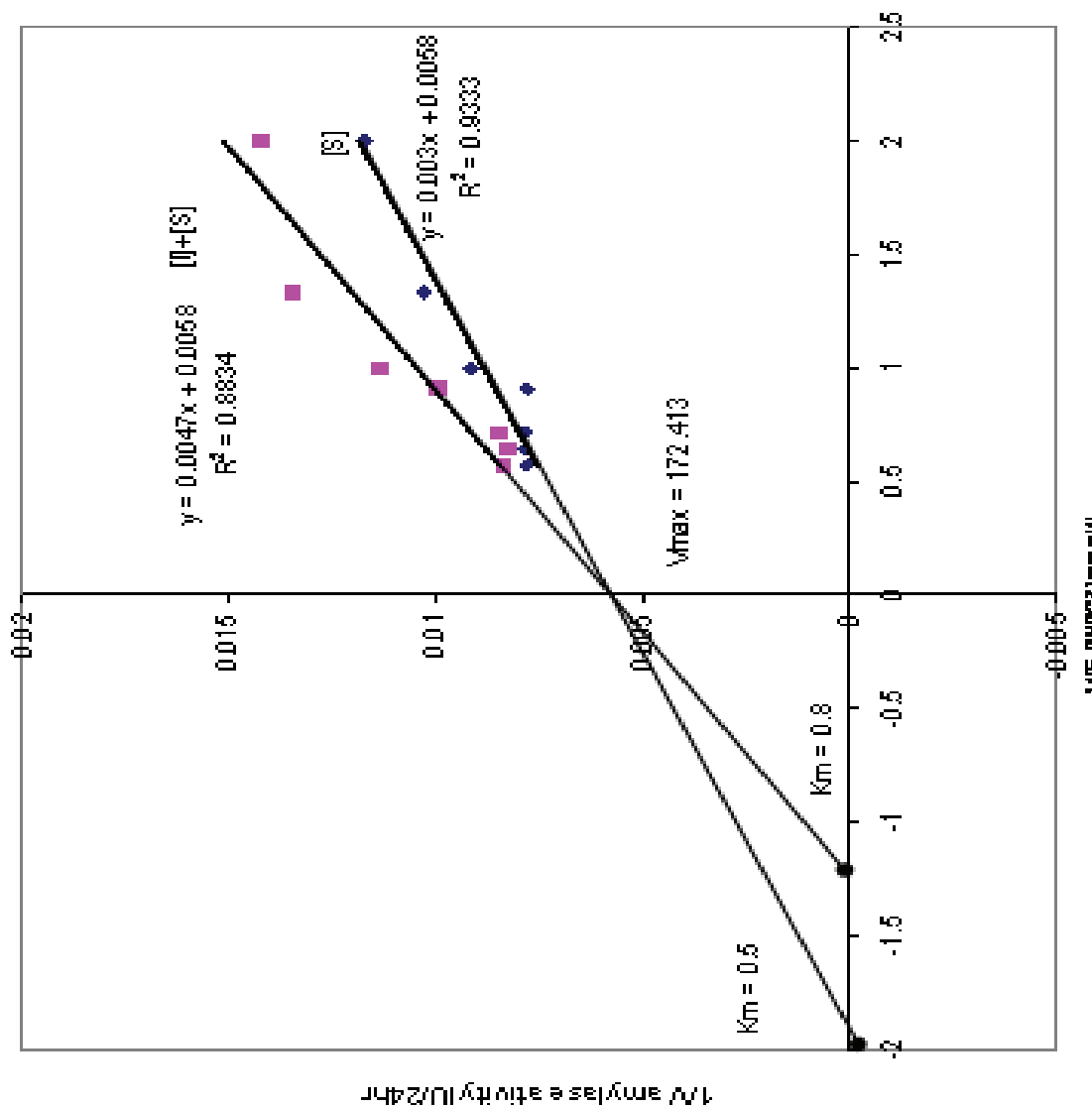


Fig-4: Lineweaver-burk plot of inhibitory effect of constant dose (15mg/ml)used of extract(A1)

**DISCUSSION:**

Gundelia tourenortii L is used as an occasional food, and its extracts have been used for prevention and treatment of liver diseases in Iran (12). The Gundelia tourenofotii extracts also have antioxidant capacities on glutathione S-transferase activity (13), in addition of that Kuub (Gundelia tourenofortii L) have hypolipemic effect on lipid profile atherosclerotic rats (14). Insulin is vital to patients with type I diabetes (diabetic ketoacidosis) can develop severely elevated blood sugar levels. This lead to increased urine glucose, which in turn leads to excessive loss of fluid and electrolytes in the urine . Lack of insulin also causes the breakdown of fat cells, with the release of ketones into the blood (15). Inhibition of amylase by this extract in D.M.I, may be like other plant for example (phaseolus acutifolius A. Gray) contain two isoforms of amylase inhibitor (16). Also there are many plants used as hypoglycemic activities (pistacia atlantica and paranychia argentea) showed significant alpha amylase inhibitory activity (17). The mechanism of the change in these analysis in D.M.I. may be related to the amount of acetone in the urine which affected by some amylase inhibitor constituents in this

plant extracts.Amount of Vmax value in the urine of D.M.I. For both [S] and [S+I] are same that is mean we have competitive

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inhibition but have different Km

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