Investigation of repressive and enhancive effects of fruit extracts on the activity of glucose-6-phophatase

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Abstract: Glucose-6-phosphatase is a key enzyme of glucose metabolic pathways. Deficiency of this enzyme leads to glycogen storage disease. This enzyme also plays a negative role in diabetes mellitus disorder in which the catalytic activity of this enzyme increases. Thus there is need for activators to enhance the activity of glucose-6-phosphatase in glycogen storage disease of type 1b while in diabetes mellitus repressors are needed to reduce its activity. Crude extracts of apricot, fig, mulberry and apple fruits were investigated for their repressive/enhancive effects on glucose-6-phosphatase *in vivo*. Albino mice were used as experimental animal. All the selected extracts showed depressive effects on glucose-6-phosphatase, which shows that all these extracts can be used as antidiabetic supplement of food. The inhibitory pattern was competitive one, which was evident from the effect of increasing dose from 1g/Kg body weight to 3g/Kg body weight for all the selected fruit extracts. However fig and apple fruit extracts showed high repressive effects for high doses as compared to apricot and mulberry fruit extracts. None of these selected fruit extracts showed enhancive effect on glucose-6-phosphatase activity. All these fruits or their extracts can be used as antidiabetic dietary supplement for diabetes mellitus.

Keywords: Glucose-6-phosphatase, glucose, glycogen storage disease, diabetes mellitus.

INTRODUCTION

Glucose-6-phosphatase (EC 3.1.3.9) plays an important role in carbohydrate metabolism. It catalyses the last step of both glycogenolysis and gluconeogenesis. Both glycogenolysis and gluconeogenesis results in the formation of glucose-6-phosphate from gluconeogenic precursors in liver and kidney, and from glycogen in liver. The glucose-6-phosphate formed is hydrolyzed by glucose-6-phophatase before being liberated as glucose into blood circulation during starvation. It not only catalyzes the hydrolysis of glucose-6-phosphate but also catalysis its resynthesizes from glucose and a phosphoryl donor. This enzyme therefore plays an important role in regulation of glucose homeostasis. Deficiency of this enzyme leads to glycogen storage disease. This is an inherited metabolic disease, characterized by poor tolerance to fasting, growth retardation and hepatomegaly resulting from accumulation of glycogen and fat in the liver. This enzyme also plays a negative role in diabetes mellitus disorder in which the catalytic activity of this enzyme increases, leading to high blood glucose level (Schaftingen and Gerin, 2012).

Deficiency of glucose-6-phophatase leads to glycogen storage disease type I (GSDI). GSDI is further categorized into GSDIa and GSDIb, the former is due to defects in catalytic unit of glucose-6-phosphatase while the latter is due to defect of glucose-6-phosphate translocase (Arion *et al.,* 1997). The estimated annual incidence of GSDI is about 1/100,000 births, representing approximately 30% of hepatic GSD with GSD1a being the most frequent type (80% of GSD1). The patients of GSDI may have fast-induced hypoglycemia which may occur rapidly in about 2 to 2.5 hours. While in new born child it may lead to hyperlactacidemia (Chen, 2000).

Cori *et al*. (1939) found that some GSDIb patients are not deficient in glucose-6-phosphatase, but different tests showed that these patients are unable to degrade glucose 6-phosphate. This was explained by Arion *et al.* (1997) by hypothesizing that glucose-6-phosphate hydrolysis required the participation of several proteins located in the endoplasmic reticulum membrane, first a catalytic unit that is capable of carrying out the enzyme activity and a glucose-6-phosphate translocase which will carry glucose-6-phosphate in to the lumen of endoplasmic reticulum, where glucose-6-phosphatase exerts its action. Glucose-6-phosphatase and glucose-6-phosphate translocase were found to be co-dependent as glucose-6-phosphatase activity is required for efficient transport of glucose-6-phosphate into the endoplasmic reticulum lumen (Cori *et al.,* 1939; Arion *et al.,* 1997).

The activity of glucose-6-phosphatase is increased in diabetic conditions, which is one of the main causes of increased glucose production. Diabetes is known as one of the important causes of death throughout the world (Can *et al*, 2004). The prevalence of diabetes in worldwide for all age-groups were estimated to be 2.8% in 2000 and in 2030 it may rise to 4.4%. This means that the total number of diabetic patients is expected to

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increase from 171 million (in 2000) to 366 million (in 2030). Insulin therapy has certain side effects like insulin allergy, autoimmunity and irregularities in metabolism, lipodystrophy and atrophy, formation of insulin antibodies, altered metabolic control, placental mobilization of insulin antibodies and other late problems that include morphological changes in kidneys and severe vascular complications (Defronzo *et al.*, 1989; Jarvinen and Koivisto, 1984; Jarvinen and Koivisto, 1986). Likewise, the oral hypoglycemic drugs have many side effects such as nausea and vomiting, agranulocytosis, dermatological reaction, jaundice, hemolytic anemias, hypersensitivity reactions and lactic acidosis (Khan and Shechter, 1991).

The inhibitors of glucose-6-phosphatase could decrease hepatic glucose output. Recently the enzyme has been identified as an important antidiabetic target. There are many compounds that inhibit the translocases of glucose-6-phosphatase system such as DIDS (4,4-di-isothiocyanostilbene-2,2-disulphonate), 2-hydroxy-5-nitrobenzaldehyde,and chlorogenic acid derivatives (Zoccoli *et al.*, 1989; Arion *et al.*, 1997; Hemmerle *et al.*, 1997; Arion *et al.*, 1998). There are few compounds, which show inhibitory activities on the glucose-6-phosphataseenzyme complex (Westergaard *et al.*, 1999; Farhanullah *et al.*, 2003). A series of 4,5,6,7-tetrahydrothienopyridines have been reported recently to be potent competitive inhibitors of the glucose-6-phosphatase (Westergaard *et al.*, 2004). There is a need to find out such compounds that competitively inhibit the glucose-6-phosphatase, hence giving an alternative to the drugs, which are inhibitors of T1-translocase.

Since ancient times, plants have been used for the treatment of diabetes mellitus in the world. The drugs from plant sources, in most cases have no toxic or other adverse effects (Rao *et al.*, 2003). According to WHO report, the anti-diabetic compounds of plant sources are important in traditional medicines. It has been estimated that 75% of world population is dependent on medicines from plant source (Wild *et al.*, 2004). Medicines of plant origin are especially famous due to their low cost, less side effects and easy availability. These plant based medicines are prepared from parts of the plant like leaves, bark, roots and seeds or they may contain whole parts of the plant. They can be taken either orally, inhaled or applied directly to the skin. The medicinal plants contain certain bioactive constituents that exhibit significant physiological action on human body. The bioactive phytochemical components are many, like alkaloids, flavonoids, terpenoids, saponin, tannins and essential oils (Weragoda 1980, Westh *et al.*, 1980). Modern preparations of drugs are mostly based on these natural compounds (Crag and Newman, 2001; Krishnaiah *et al.*, 2007).

This study was aimed to find out promoters from plant origin, capable of enhancing the activities of glucose-6-phosphatase in GSD type Ib disorders and inhibitors capable of slowing down the effects of glucose-6-phosphatase activity in diabetes mellitus disorders.

MATERIALS AND METHODS

Glucose-6-phosphatase was purchased from Sigma Aldrich while Potassium dihydrogen phosphate, Tris HCl Buffer (0.5M, Sterile pH 6.8), Glucose-6-phosphate disodium salt hydrate, Ammonium molybdate, Sodium sulfite (anhydrous), Sodium bisulfate, 1-Amino-2-naphthol-4-sulfonic acid were purchased from Panreac Quimica Sa (Spain). Mice feed was purchased from local market. The reagents were of high purity grade and were used as such without further purification.

Albino mice were used as experimental animals in this study. Four couple albino mice were kept at one time in well ventilated and wide chambers in animal house University of Malakand for three months for breeding. The animals were fed on a well-balanced diet. Thirty-nine albino mice of either sex weighing 25-40grams were used as experimental animals.

Apple, apricot, mulberry and fig fruits were collected from their natural growing localities of Malakand division, Khyber Pakhtunkhwa. The fruits were botanically authenticated and samples were stored in the herbarium of University of Malakand.

The selected fruits were cleaned, shade dried and coarsely grounded. The powdered materials were soaked in 70% methanol for 3 days with occasional shaking. The soaked materials were filtered through a muslin cloth. The procedure was repeated three times and the combined filtrates were evaporated on rotary evaporator under reduced pressure and a thick solution of each fruit extract was obtained. The thick solutions were transferred to a tray, placed in a water bath and the remaining solvent in each crude extract was evaporated.

The experiments were carried out to assess the effect of crude extracts of Apple, apricot, mulberry and fig on liver glucose-6-phosphatase activities. The selected thirty-nine mice were divided into five groups. Details of the groups are given as under

Group 1: Control group, received no dose.
Group 2: Received fig fruit extract.
Group 3: Received mulberry fruit extract.
Group 4: Received apricot fruit extract.
Group 5: Received apple fruit extract.

Each group from 2 to 5 was further divided into 3 sub-groups and were arbitrary named as A, B, C etc. The replicates of each group were given doses of 1, 2 and 3g/Kg body weight.

Solutions of each crude extract were prepared having concentration 1, 2 and 3g/10ml. Amount of dose given was calculated for each group on the basis of weight of animal and rate of dose given to each group. Administration of dose was done through feeding tubes in liquid form. The dose was given to each mouse daily for three weeks.

On 22nd day of feeding, mice of all the groups were killed. Their livers were collected and stored in ice cold water in individual flasks. Just before the measurement of enzyme activity liver homogenates were prepared with ice cold water. The homogenates were then placed in shaker for 1 hour to get the enzyme extract; temperature of shaker was maintained at 0 C by the continuous addition of ice. The supernatant obtained was filtered and was used as enzyme source.

For calculating enzyme activity, 1ml of liver homogenate, 1.5ml tris (hydroxy methyl) amino methane buffer of pH 6.7 and 2.5ml of.01M glucose-6-phosphate were incubated for 1 hour at 33πC. After 1hour 1ml 10% trichloroacetic acid was added to the reaction mixture and inorganic phosphate released during the reaction was measured by method of Fiske and Subbarow (1925). Liver homogenate (1ml) of each sample were taken in separate flasks and to each of these, 2.5ml glucose-6-phosphate and 1.5ml of buffer were added. The mixtures were incubated for one hour at 38°C; the reaction was stopped by adding 1ml 10 percent trichloroacetic acid. The samples were centrifuged, and supernatant were used for measuring the enzymatic activity.

From each liver extract 5ml were taken in a flask; to it 4 ml each of 0.25 percent aminonaphthol sulphonic acid and 2.5% ammonium molybdate made in sulfuric acid were added. The total volume was made up to 70 ml by adding distilled water with gentle shaking. The amount of inorganic phosphate released was measured using UV-Visible spectrophotometer at 650nm wavelength. The same procedure was used for the preparation of standard. However instead of liver extract the purified glucose-6-phosphatase was added. The concentration of inorganic phosphate in each sample was determined from comparison with standard phosphate calibration curve as given below.

Working standards of different concentrations ranging from 0.1 to 0.71 mg were prepared in 10N sulfuric acid. Colour was developed using the procedure mentioned above and absorbance was measured using UV-Visible spectrophotometer at wave length of 650 nm. Curve was plotted from the data obtained.

RESULTS

A number of factors like enzyme concentration, substrate concentration, product concentration, temperature, pH, availability of coenzymes, and the presence of activators or inhibitors affect the enzyme catalytic activities. The presence of repressors or enhancers will also affect the enzyme activity by altering the enzyme concentration in the cell. The present study was about the investigation of the repressive or enhancive effect of fruit extracts on the activity of glucose-6-phophatase. Four fruits; apricot, fig, apple and mulberry were collected from their natural growing localities of Malakand division, Khyber Pakhthunkhwa. The effect of the selected fruit extracts on enzyme activity are shown in tables 1, 2, 3 and 4. Graphically the results are given in figures 1 to 4.

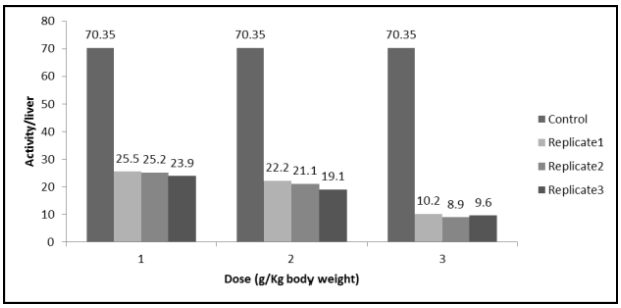

Fig. 1: Effect of apricot fruit crude extract on glucose-6-phosphatase activity

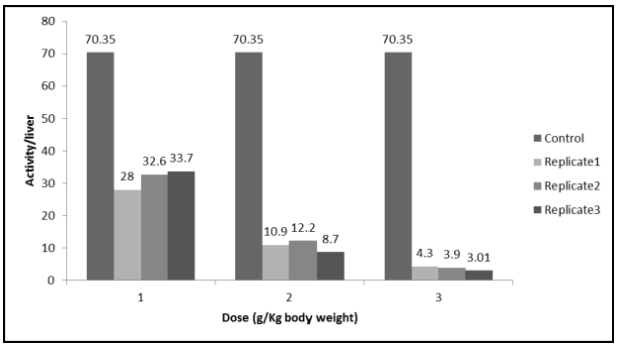

Fig. 2: Effect of fig fruit crude extract on glucose-6-phosphatase activity

DISCUSSION

Repressive effects of selected fruit extracts on the activity of glucose-6-phosphatase

Enzyme inhibition and repression both reduce the enzyme velocity. Yet the mechanisms are different. The inhibitor act on enzyme directly and the inhibitory affects are noticed as soon as inhibitor is added. The number of enzyme molecules is not changed by inhibitor. On the contrary, repressors act at gene level and their effect is noticeable only after a lag period of hours or days. The number of enzyme molecules is reduced in presence of repressor. As mentioned in the previous sections that glucose-6-phosphatase is an important enzyme of glucose metabolic pathways. Glucose-6-phosphate formed in glycogenolysis and gluconeogenesis is hydrolyzed by glucose-6-phophatase to liberate glucose into blood circulation during starvation. In diabetes mellitus disorder

the catalytic activity of this enzyme increases, which leads to high blood glucose level. Inhibitors/repressors are needed to reduce the activities of this enzyme. Any component capable of reducing its activity is called antidiabetic. All these selected fruits extract showed inhibitory effects on glucose-6-phosphatase activities. The individual effect of each fruit extract glucose-6-phosphatase activity is described below.

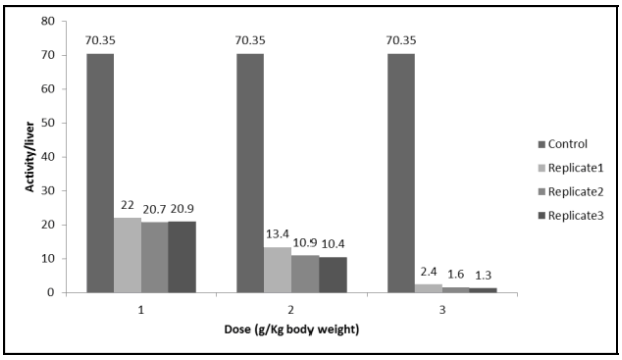

Fig. 3: Effect of apple fruit crude extract on glucose-6-phosphatase activity

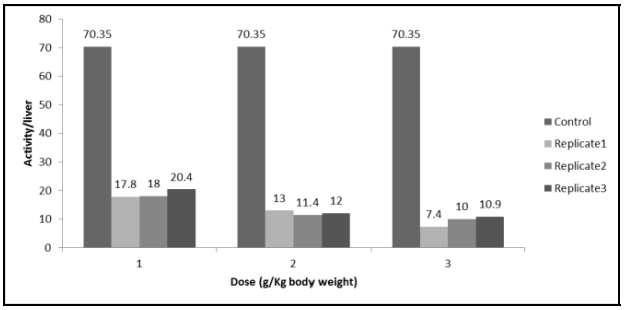

Fig. 4: Effect of mulberry fruit crude extract on glucose-6-phosphatase activity

Effect of apricot fruit crude extract on glucose-6-phosphatase activity

Table 1 shows the effect of apricot crude extract on glucose-6-phosphatase activity in mice liver. It is evident from the table that the apricot crude causes a significant depression in glucose-6-phosphatase activity per liver. The depression in the activity of this enzyme shows that there are certain factors present in the extract that may causes a reduction in blood glucose. If this also occurs in diabetic patients treated with apricot crude extracts, it might be an important diet supplement in reducing the fasting blood glucose level and its excretion in urine. The increase in dose of crude extract causes a significant depression in glucose-6-phosphatase activity. The highest depression in the enzymatic activity was observed for 3g/Kg body weight dose of the extract. This was due to high concentration of the active inhibitors of glucose-6-phosphatase present in the extracts. The results are shown graphically in fig. 1. The inhibitory pattern is competitive one, which is evident from the effect of increasing dose from 1g/Kg body weight to 3g/Kg body weight.

Effect of fig fruit crude extract on glucose-6-phosphatase activity

Table 2 shows the effects of fig fruit crude extracts on glucose-6-phosphatase activity. Nearly the same trend as that for apricot crude extract was observed. However with the increase in dose in case of fig fruit extract up to 3g/Kg body weight the effect was more pronounced as compared to apricot extract. This was due to synergistic inhibitory effect of the active on glucose-6-phophatase. The results are graphically shown in fig. 2. The inhibition pattern seems to be competitive in which the inhibitor binds free enzyme and compete for active site of enzyme with substrate. The increase in concentration of inhibitor present in extract causes a decline in the reaction velocity.

Effect of apple fruit crude extract on glucose-6-phosphatase activity

The apple crude extract showed an inhibitory effect on glucose-6-phosphatase. This is evident from table 3 and fig. 3. The effect was more pronounced as compared to fig and apricot fruit extracts. This implies that the concentrations of active inhibitors are higher in apple extracts or there are powerful inhibitors of glucose-6-phophatase in the apple extract. Comparatively high inhibition of the glucose-6-phophatase for 1g/Kg body weight implies that the inhibitors present in apple fruit crude extract are powerful inhibitors.

Effect of mulberry fruit crude extract on glucose-6-phosphatase activity

The inhibitory pattern of mulberry fruit extracts found was somewhat similar to apricot fruit extract (table 4). The inhibitory effect is more pronounced for 1g/Kg body weight as compared to the rest of the three extracts. However the inhibitory effect for 2 and 3g/Kg body weight doses were quantitatively similar to that of apricot fruit extract. The results are shown graphically in fig. 4.

Enhancive effects of selected fruit extracts on the activity of glucose-6-phosphatase

Glycogen storage disease is an inherited glucose metabolism disorder characterized by poor tolerance to fasting, growth retardation and hepatomegaly resulting from accumulation of glycogen and fat in the liver. The disorder is caused by deficiency of glucose-6-phosphatase. As earlier mentioned there are two types of glycogen storage disease, GSDIa is due to defects in catalytic unit of glucose-6-phosphatase while GSDIb is due to defect of glucose-6-phosphate translocase. According to Cori *et al* . (1939) GSDIb patients are not deficient in glucose-6-phosphatase, but different tests showed that these patients are unable to degrade glucose 6-phosphate.Glucose-6-phosphatase and glucose-6-phosphate translocase are co-dependent as glucose-6-phosphatase activity is required for efficient transport of glucose-6-phosphate into the endoplasmic reticulum lumen. Glucose-6-phosphate translocase is responsible for

Table 1: The effect of apricot fruit extract on glucose-6-phosphatase activity of Mice Liver

S No	Sub group	Replicates	Extract dose (g/kg body weight)	Total activity per liver	Average	Standard deviation
1.	A	A1	1	25.5		
		A2		25.2	24.87	±0.85
		A3		23.9		
		Control		70.35		
2.	B	B1	2	22.2		
		B2		21.1	20.80	±1.57
		B3		19.1		
		Control		70.35		
3.	C	C1	3	10.2		
		C2		8.9	9.56	±0.65
		C3		9.6		
		Control		70.35		

Table 2: The effect of fig fruit extract on glucose-6-phosphatase activity of Mice Liver

S. No	Sub group	Replicates	Extract dose (g/kg body weight)	Total activity per liver	Average	Standard deviation
1.	D	D1	1	28.0		
		D2		32.6	31.43	±3.02
		D3		33.7		
		Control		70.35		
2.	E	E1	2	10.9		
		E2		12.2	10.59	±1.77
		E3		8.7		
		Control		70.35		
3.	F	F1	3	4.3		
		F2		3.9	3.74	±0.66
		F3		3.01		
		Control		70.35		

Table 3: The effect of apple crude extract on glucose-6-phosphatase activity of Mice Liver

S. No	Sub group	Replicates	Extract dose (g/kg body weight)	Total activity per liver	Average	Standard deviation
1.	R	R1	1	22.0		
		R2		20.7	21.20	±0.69
		R3		20.9		
		Control		70.35		
2.	S	S1	2	13.4		
		S2		10.9	11.57	±1.61
		S3		10.4		
		Control		70.35		
3.	T	T1	3	2.4		
		T2		1.6	1.77	±0.57
		T3		1.3		
		Control		70.35		

the transport for glucose-6-phosphate phosphate into the endoplasmic reticulum lumen.

The study was aimed to find out such carriers from fruit origin capable of glucose-6-phosphate into the endoplasmic reticulum lumen that would enhance the activity of glucose-6-phosphatase and thus would be the potential food supplement for the control of glycogen storage disease in GSDIb type disorders. However “no such activities were observed for the selected fruit extracts”. Thus it is inferred that the selected fruits should not be used by patients suffering from glycogen storage disorders as it will further repress the activity of glucose-6-phophatase and would worsen the problem.

Table 4: The effect of mulberry fruit extract on glucose-6-phosphatase activity of Mice Liver

S. No	Sub group	Replicates	Extract dose (g/kg body weight)	Total activity per liver	Average	Standard deviation
1.	X	X1	1	17.8		
		X2		18.0	18.73	±1.45
		X3		20.4		
		Control		70.35		
2.	Y	Y1	2	13.0		
		Y2		11.4	12.13	±0.81
		Y3		12.0		
		Control		70.35		
3.	Z	Z1	3	7.4		
		Z2		10.0	9.43	±1.82
		Z3		10.9		
		Control		70.35		

CONCLUSION

In this study apricot, fig, mulberry and apple fruits extracts were investigated for their repressive/enhancive effects on glucose-6-phosphatase activity. All these selected fruit extracts showed inhibitory effects on the activity of glucose-6-phosphatase. Thus these fruit extracts can be used as antidiabetic supplement for clinical medicines, as in diabetes mellitus glucose-6-phosphatase plays a negative role and repressors are needed to slow down its activity. The increase in dose from 1g/Kg body weight to 3g/Kg body weight results in high inhibition for all the selected fruit extract. No enhancive effect was observed for any of the crude fruit extracts. Thus it is inferred from the results that these fruits can be used as antidiabetic but in case of glycogen storage disease of type 1b these fruits or their extracts should be used with care.

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