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Acute and Chronic Effects of Phenobarbital on the Glucose 6 Phosphatase Enzyme, Glucose and Glycogen Concentration in Rat Liver

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ABSTRACT

Phenobarbital is used as an antiepileptic drug. It activates many metabolizing enzymes in the liver. Accumulation and consumption of phenobarbital is very high in the brain, kidney and liver. .It also has the ability to distribute in tissues and body fluids. . Glucose-6-phosphatase plays an important role in stabilizing blood sugar. Its main physiological role in the liver is to catalyse the dephosphorilation of G6P. Material and methods: In this study, to determine the chronic effects of phenobarbital on the glucose-6-phosphatase enzyme activity and its effect on the amount of glucose and glycogen, 45 male rats of the wistar strain [200-250 g] were used. Blood sample connection was performed through Decapitation method. Measuring glucose-6-phosphatase enzyme activity, Blood serum glucose and hepatic glycogen content in rats was performed respectively according to the Bell and Doisy method, ortotholoidin method and Mendel method. Results: The amount of glucose, liver glycogen and Glucose 6 phosphatase activity in the group of animal's interaperitoneally injected with 100 mg PB after 48 and 72 hours showed a significant difference with the control samples. [P<0/05]. Long term injection of 40 and 60 mg PB after 10 days showed a significant decrease in the activity of glucose-6-phosphatase and glucose concentration and after 15 and 60 days showed a significant difference with the control samples. Conclution: by increasing time and dose, glucose decreases and glycogen increases. On the other hand, following a decrease in glucose and glucose-6-phosphatase enzyme activity, Glucokinase activity increases and subsequently gluconeogenesis is activated.

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INTRODUCTION

Phenobarbital reversibly reduces the activity of excitable tissues. Its main effect is on the microsomal metabolizing system. . Enzyme activity enhances, protein and fat in the smooth muscles ER, stimulating enzymes responsible for the metabolism of the body's natural components such as bilirubin and is used in neonatal hypobilirubin. [11, 32] Phenobarbital is used as an antiepileptic drug. [18] It activates many metabolizing enzymes in the liver.[19] Long-term utilization of this substance leads to chronic damage to the liver. Phenotypic Symptoms of its consumption are disorder, sleepiness, depression and anxiety. [6] Most of its activity is in the ER of liver and kidney. Accumulation and consumption of phenobarbital is very high in the brain, kidney and liver.It also has the ability to distribute in tissues and body fluids. [12]

Some of Phenobarbital consumption impacts is a reduction in the concentration of some carcinogens which are by products of smoking. It is also important to note that PB reduces smoking based bladder carcinoma while has a converse impact on smoking based lung cancer.[31] PB cause liver tumors and its activity is restricted to CAR receptor [Constitutive active/androstane].[33] Among the Possible mechanisms for the effect of phenobarbital oxidative stress and damage to cellular DNA, has been reported.[27]

NF-Bκ - transcription factor which is activated by oxidative stress, leads to proliferation and apoptosis of liver cells.[20] This factor is readily activated by PB.[4] Due to the activation of NF-Bκ oxidative stress, thus antioxidants can prevent this activation.[10] Vitamin E prohibits the PB induced intraction of NF-Bκ and cellular DNA.[1,20] But ,had no effect on cell growth parameters produced due to PB consumption and Only prevent the induction of BκNF- activity.[20]

Glucose-6-phosphatase plays an important role in stabilizing blood sugar By trasfering glucose-6-phosphate intermediates into ER.[9,12] The main physiological role of G6Pase in the liver is to catalyse the

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dephosphorilation of G6P .[25] The enzyme is active in PH=6-7 and zinc ions, flurizin [26] and ferritin [23] are its main inhibitors. Activity of glucose-6-phosphate is related to hormones and diet.[9] It has Highest activity in rat liver lobules surrounding areas and Starvation increases 2 to 3 times its activity in central and peripheral lobules of rat liver .[15] the glucose molecules which are transferred into the liver,are phosphorilated to G6P by glucokinase. Hormones such as glucagon and insulin regulate the activity of glucokinase. Immunological [22, 25] researches have demonstrated that glucose-6-phosphate dehydrogenase deficiency can be dangerous for diabetics. [5] Low activity of this enzyme is associated with glycogen storage disease [25]. Untreated diabetes increases the activity of glucokinas [2, 30] and activity will return to normal after treatment [16, 30].

MATERIAL AND METHODS

Material:

All the chemical materials were prepared from Merck Germany.

Methods:

Animals:

Male rats of the wistar strain [200-250 g] were kept in cages in 25°C. 45 rats were injected with 50-100 mg/kg Phenobarbital per day. The desired parameters were measured in 24-48 and 72 houres after injection.

To determine the chronic effects of phenobarbital on the glucose-6-phosphatase enzyme activity and its effect on the amount of glucose and glycogen[long-term] 75 rats were divided into 15 groups of five numbers. Rats of group 1 were injected with physiologic serum[control group] and groups 2,3,4,5 were injected intrapenitoally with 10,20,40 and 60 ml/kg PB respectively. After 10, 15 and 60 days, the mice were dissected and required measured factors were measured.

Dissection and sampling:

blood sample conection was performed through Decapitation method by guillotine. This method is fast and a maximum amount of animal blood serum can be obtained after centrifugation.Blood serumshoud be transferred to the test tube , quickly to avoid blood clotting.

Keep the liver:

Immediately after killing the animals, the liver was removed and washed in cold physiologic serum and was dewatered. Part of the liver removed and cut into small pieces [all steps were performed at 4°C . Then 8 ml/gr of 0/25 M sucrose solution was added to the container content. Sucrose provide a neutral environment and is isotonic to the cytoplasm and cytosol. Measuring Blood serum glucose in rats was performed according to the ortotholoidin method. Ortotholoidin react with aldehyde groupe of gloucose and produces an equilibrium mixture of glycosylamine and related shift base[28] green colore and a measurable wavelength of 630 nm.[34] Hepatic glycogen content was measured according to the method of Mandel *et al*[Mandel, etal, 1954]. This method, glycogen in the presence of hot and concentrate sulfuric acid is converted to 5-hydroxymethyl furfural . Then the latter substrate reacts with an intermediate produced from the combination of glucose and methyl furfural and turn into pink. the glycogen content can be measured from Light absorption so that the pink color intensity is in direct proportion to the glucose concentration.[24] Glucose 6-phosphate is enzyme activity is higher in the liver and kidney and located in microsoms. This enzyme is a main factor of carbohydrate metabolism and its activity is measured usually by measured of inorganic phosphates resulting from hydrolysis of glucose-6-phosphate. Enzyme activity measurment of glucose-6-phosphate was performed according to the method of Bell and Doicy[Bell and Doisy] [14].

Results:

Glucose 6 phosphatase activity , glucose concentration and liver glycogen of rat After receiving 50 and 100 mg/kg phenobarbital at 24, 48 and 72 hours is given in tables 1,3,5 respectively. Tables 2,4,6 show this parameters after 10,20,40,60 mg/kg Phenobarbital intraperitoneally injected at days 10,15,60.

Table 1: Changes of specific activity of the enzyme glucose-6-phosphatase after IP injection of phenobarbital in rats [short-term]

Specific activity[5 days] [nmol/min/mg]	Specific activity[3days] [nmol/min/mg]	Specific activity[1 day] [nmol/min/mg]	specific activity of control nmol/min/mg]	Day concentration
1.482[%68.1] ± 0.51	2.345[%49.5] ± 0.75	3.35[%27.8] ± 1.08	4.65 ± 0.75	50 mg/kg
1.455[%68.7] ± 0.7	2.21[%52.5] ± 0.16	2.178[%53.2] ± 0.68	4.65 ± 0.75	mg/kg100

Results at the level of 0/05 percent are meaningful.
Numbers in parentheses are percent of changes compared with control.

Table 2: Changes in specific activity of the enzyme glucose-6-phosphate after IP injection of phenobarbital in rats [long-term].

Specific activity[60 days] [nmol/min/mg]	Specific activity[15 days] [nmol/min/mg]	Specific activity[10 days] [nmol/min/mg]	specific activity of control [nmol/min/mg]	Day concentration
2.06[%55.7] ± 1	2.515[%45.9] ± 2.29	3.445[%25.9] ± 0.54	4.65± 0.75	10 mg/kg
2.07[%55.4] ± 0.63	2.458[%47.1] ± 0.8	*3.250[%30.1] ± 0.52	4.65 ±0.75	20 mg/kg
1.9[%59.1] ± 1.56	2.43[%47.7] ± 1.18	*2.774[%40.3] ± 0.99	4.65 ± 0.75	40 mg/kg
*3.74[%19.6] ± 0.91	2.289[%50.7]± 0.69	*3.887[%16.4] ± 1.38	4.65 ± 0.75	60 mg/kg

Results at the level of 0/05 percent are meaningful.
Numbers in parentheses are percent of changes compared with control

Table 3: Changes of glucose concentration of blood after IP injection of phenobarbital in rats [short-term].

Specific activity[3 days] [ml/100mg]	Specific activity[2 days] [ml/100mg]	Specific activity[1 day] [ml/100mg]	specific activity of control [ml/100mg]	Day concentration
139[%6.08] ± 2.95	140[%5.4] ± 9.75	144[%2.7]± 9.95	148 ± 8.03	50 mg/kg
*135[%8.78] ± 7.31	*134[%9.46] ± 6.24	135[%8.78]± 8.75	148± 8.03	100 mg/kg

Results at the level of 0/05 percent are meaningful.
Numbers in parentheses are percent of changes compared with control

Table 4: Changes of glucose concentration of blood after IP injection of phenobarbital in rats [long-term].

Specific activity[60 days] [nmol/min/mg]	Specific activity[15 days] [nmol/min/mg]	Specific activity[10 days] [nmol/min/mg]	specific activity of control [nmol/min/mg]	Day Concentration
*124[%16.2]± 13.71	134[%9.46] ± 13.83	146[%1.35] ± 8.21	148± 8.03	10 mg/kg
*124.8[%15.7] ± 8.23	*130[%12.2] ± 7.52	146[%1.35] ± 8.57	148 ± 8.03	20 mg/kg
*115[%22.3] ± 11.05	130[%11.5] ± 9.14	*134[%9.46] ± 7.1	148 ± 8.03	40 mg/kg
*122[%17.6] ± 6.08	*127[%13.85] ± 6.38	*132[%10.8] ± 5.92	148 ± 8.03	60 mg/kg

Results at the level of 0/05 percent are meaningful.
Numbers in parentheses are percent of changes compared with control

Table 5: concentration changes of liver glycogen after IP injection of phenobarbital in rats [short-term].

Specific activity[3 days] [mg/kg]	Specific activity[2 days] [mg/kg]	Specific activity[1 day] [mg/kg]	specific activity of control [mg/kg]	Day concentration
32.5[%6.2] ± 2.15	31.7[%3.59] ± 1.67	30.7[%0.33]± 2.16	30.6 ± 1.19	50 mg/kg
33.1[%8.2] ± 3.01	*32.9 [%7.5] ± 2.25	30.9[%0.98] ± 1.23	30.6 ± 1.19	100 mg/kg

Results at the level of 0/05 percent are meaningful..
Numbers in parentheses are percent of changes compared with control

Table 6: *concentration* changes of liver glycogen after IP injection of phenobarbital in rats [long-term].

Specific activity[60 days] [nmol/min/mg]	Specific activity[15 days] [nmol/min/mg]	Specific activity[10 days] [nmol/min/mg]	specific activity of control [nmol/min/mg]	Day concentration
32.3[%5.56] ± 3.05	31.5[%2.94] ± 2.6	30.7[%0.33] ± 1.23	30.6±1.19	10 mg/kg
*32.5[%6.21] ± 2.25	*32.8[%7.19] ± 2.31	30.9[%0.98] ± 1.27	30.6± 1.19	20 mg/kg
*33.4[% 9.15] ± 3.4	32.5[%6.21] ± 2.22	31.1[%1.6] ± 2.16	30.6 ± 1.19	40 mg/kg
*33.8[%10.45] ± 3.62	*32.8[%7.19]± 3.01	31.2[%1.96] ± 2	30.6 ± 1.19	60 mg/kg

*Results at the level of 0/05 percent are meaningful.
Numbers in parentheses are percent of changes compared with control.

The amount of glucose in the group of animals interaperitoneally injected with 100 mg PB showed a significant difference with the control samples.

Glycogen injection did not have any effect after 24 hours. However, after 48 to 72 hours a significant increase was shown.[$p<0.05$]

Long term injection of 10,20,40 and 60 mg/kg/day of PB for 10 , 15 and 60 days also evidenced a decrease in the activity of G6Pase along with increase in dose and time.

Consumption of PB for a period of 10 days showed a significant decrease in the activity of this enzyme. [$p<0.05$]

Daily injection of 40 mg PB to rats also showed a significant difference with the control samples. The amount of glucose with injection of 40 and 60 mg PB showed a significant reduction after 10 days.[$P<0/05$]. 20 and 60 mg concentrations after 15 days and total concentrations after 60 days showed a significant difference with the control samples.

Discussion and conclusion:

Liver is the only organ that controls blood glucose levels because it has phosphorilation and dephosphorilation enzymes of hexoses[especially glucose] . Therefore it plays an important role in the metabolism of carbohydrates in the body. Control of Carbohydrate metabolism in the body is done by the balance between the activity of the enzymes hexokinase [especially Glucokinase] and glucose-6-phosphatase . . Thus , anything that affects the activity of one or both enzymes, alters carbohydrate metabolism [7]. Phenobarbital affects the liver enzymes and reduces impacts of other drugs . Also, it stimulates the natural

enzymes that are responsible for metabolism [such as bilirubin] and hepatic microsomal enzymes that play a vital role in the metabolism of drugs .[21]. Following Short-term injection of 50 and 100 mg/ kg phenobarbital at 24, 48 and 72 hours, with an increasing time and dose, the activity of the enzyme glucose-6-phosphatase decreased. Usage of phenobarbital had no effect in the first 24 hours, but the amount of 100 mg after 48 and 72 hours showed a reduction effect. liver tissue influenced by Phenobarbital showed a change in color, morphology and histology. Long-term injection of 10, 20,40 and 60 mg/kg /day phenobarbital for 10, 15 and 60 days showed a reduction in the glucose-6-phosphatase enzyme activity while increasing time and dose of PB . Phenobarbital causes changes in the liver tissue and reduces synthesis of glucose-6-phosphate in the endoplasmic reticulum .[3,17] Inhibitory effect of PB on glucose-6-phosphatase reduces the glucose levels . Phenobarbital treatment enhances insulin-mediated glucose metabolism and improves lipid metabolism in the diabetic rat.As well, there is a strong association between glucose metabolism and hepatic microsomal enzymes activity [29]. Hepatic gluconeogenesis is highly dependent on glucose-6-phosphatase enzyme activity [6,18] Although the consumption of phenobarbital reduces cocentration of glucose and glucose-6-phosphate, But glyconeogenesis substances contained Pyrovic acid, lactic acid and some amino acids will be converted to glucose-6-phosphate and excess of G6P will inter pathway of glycogen synthesis and will increase glycogen concentration. So,an increase in glycogen concentration is not because of an increase in glucose concentration.In brief, by increasing time and dose, glucose decreases and glycogen increases.On the other hand, following a decrease in glucose and glucose-6-phosphatase enzyme activity, Glucokinase activity increases and subsequently gluconeogenesis is activated.

Conclusion:

By increasing time and dose, glucose decreases and glycogen increases.On the other hand, following a decrease in glucose and glucose-6-phosphatase enzyme activity, Glucokinase activity increases and subsequently gluconeogenesis is activated.

1- The effect of phenobarbital on liver cells, decrease the activity of the enzyme glucose-6-phosphatase and subsequently the blood sugar level is reduced.

2- Phenobarbital cause an increased in hepatic glycogen.

3- Low doses of phenobarbital can be used to help people with diabetes.

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