Research Paper

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Therapeutic Effect of Aqueous Extract of Vitis Vinifera L. Against Thyrotoxicosis Induced by Levothyroxine Sodium in Rabbit Females

Chemistry

KEYWORDS : Vitis Vinifera L., Levo thyroxine sodium, LDH, ACP, ALP.

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ABSTRACT

To investigat the hepatoprotective potential of aqueous grape leaf extract on levothyroxine sodium induced liver toxicity in femals of rabbit. Liver injury was induced by levothyroxine sodium (5 µg) on the first day of experiment. Vitis vinifera L.leaf extract (100mg/ml) was administered orally daily for two months. The animals were sacrificed after two months and the hepatoprotective activity was assessed by using various biochemical parameters. In levothyroxine sodium treated femals of rabbit , the lactate dehydrogenase(LDH), acid phosphatase (ACP), and alkaline phosphatase (ALP) levels were significantly increased. The Vitis vinifera L.leaf extract administration therapy the toxic effect of levothyroxine sodium on the LDH, ACP, and ALP.

Introduction

Vitis vinifera L. (Family: Vitaceae) is one of the most popular and the world's largest fruit crop[1]. Reviewing the current literature, it was found that Vitis vinifera L. contains many chemical constituents viz, phenolic acids, flavonoids, anthocyanins, proanthocyyanidins, sugars, sterols, amino acids, and minerals [2]. Natural antioxidants have gained considerable interest in recent years for their role in preventing the auto oxidation of fats; Vitis vinifera L. plant is considered as a natural antioxidant source [3].

Vitis vinifera L. is considered as an economic plant, whose several cultivars which are grown for their edible fruits, the source of raisins, wines, sultanas and currents [4]. Grape are also used as demulcent, laxative, refrigerant, stomachic, diuretic and cooling. Moreover, it is useful in bilious dyspepsia, haemorrhage, dysuria, in chronic bronchitis heart disease and gout[5].

Lactate dehydrogenase(LDH, EC 1.1.1.27):

is ahydrogen transfer enzyme that catalyses the oxidant of L-lactate to pyruvate with nicotinamide- dinucleotide (NAD)+ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favours the revers reaction, namly the reduction of pyruvate(P) to lactate(L) [6]:

$$\text{L-lactate} + NAD^+ \underset{\text{Ph 7.4-7.8}}{\overset{\text{LDH . PH 8.8-9.8}}{\rightleftharpoons}} \text{Pyruvate} + NADH + H^+$$

Acid and alkaline phosphatase: have been traditionally classified as being acid and alkaline, due to their optimum pH activity, above pH 7.0 or below pH 7.0 [7]. Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1)are enzymes that catalyze the removal of inorganic phosphate (orthophosphate)from organic phosphate esters, in acidic and alkaline media respectively. These phosphatase are ubiquitous in plants, animals and microorganisms[8].

Thyroxine:

is the main hormone secreted into the bloodstream by the thyroid gland. It is inactive and most of it converted to an active from called triiodothyronine by organs such as the liver and kidneys. Throid hormones play vital roles in regulating the body's metabolic rate, heart and digestive functions, muscle control, brain development and maintenance of bones [9].

The aim of this study was to evaluate effect of aqueous extract of Vitis vinifera L. leaves on LDH, ACP and ALP levels in rabbit females have thyrotoxicosis induced by levothyroxine sodium.

Materials and methods

Samples of whole dried Vitis vinifera L. were brought from Iraqi market in Baghdad, then leaves of the plant were isolated and kept in airtight glass containers till the time of the experiment. Then the dried plant leaves were ground to fine powder. 25 gm of the powder, mixed with 250 mLof distilled water and were incubated for 3hrs at (60)°C then incubated overnight at room temperature. Suspension was then filtered. Water extracts were prepared daily just before administration orally to the experimental animals in a dose of (5 mL rabbit of (1.5-2)kg) .

Preparation of levothyroxine sodium:

Fresh solution of levothyroxine sodium was prepared (tablet dissolve in water or food) just before feeding. For the animals given the levothyroxine sodium (50µg/each rabbit).

Experimental animals: Eighteen

female oryciolagus cuniculus rabbit (1.5-2 Kg. each) were kindly supplied by city of Medicine, for the period from September 2012 to May 2013. And were used in this research. Rabbit were maintained with free access to water and diet (containing multivitamins, vegetables, and wheat). Experimental animals were divided into two groups (9 rabbit each):

1- Control group: rabbit were orally administered (using a feeding solution) with a daily dose of 5 mL distilled water for 2 months;
2- Plant- treated levothyroxine sodium group: : 50µg of levothyroxine sodium was orally administered daily to each rabbit in this group for 1 month, then 5 mL of the plant extract (100 mg/mL) was orally administered daily to each rabbit in this group for 2 month.

Blood sampling: Blood samples were collected from the heart of rabbit using heparinized capillary tubes. Serum was separated from blood samples, then frozen until used.

The activity of enzymes (LDH, ACP, and ALP) in the serum were determined according to method of King J(1965), Bergmere H. U., et al(1974) and Roy A.V. (1970) respectively (10-12).

Statistical analysis: All statistical analysis of the study were done using SPSS version 15.0 for Windows (statistical Package for Social Science, Inc., Chicago, IL, USA).

Descriptive analysis was used to show the mean ± standard deviation of variables. The significance of difference between mean values was estimated by Student T- test. The probability $p < 0.05$ = significant.

Results and discussion

Results presented in table 1 indicate that the levels of diagnostic marker enzymes namely LDH, ACP, and ALP levels were significantly ($p<0.001$) increased in levothyroxine sodium treated femals of rabbit when compared with normal rabbit. However, treatment of rabbit female with Vitis vinifera L.significantly decreased ($p<0.001$) diagnostic marker enzymes like LDH, ACP, and ALP levels when compared to L. thyroxine sodium treated rabbit females.

Table(1): Illustrate levels of LDH, ACP and ALP in blood rabbit females.

	Control C	L.Thyroxine sodium G1	L.Thyroxine sodium +Plant extract 1 month G2	L.Thyroxine sodium +Plant extract 2 month G3	p-value				
	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	G1vs.C	G2vs.C	G3vs.C		G1 vs.G2
LDH U/L	147.8± 2.02	173.5 ± 2.03	160.4± 0.44	150.3 ± 0.53	0.0001**	0.0001**		0.001*	0.0001**
ACP U/L	6.22±0.11	7.34± 0.11	6.52± 0.12	4.9 ± 0.18	0.0001**	N.S		0.0001**	0.001*
ALP U/L	17.10±0.53	22.2±0.49	20.1± 0.38	8.7±0.33	0.0001**	0.0001**		0.0001**	0.05*

SEM: Mean standard error, P=<0.0001 (highly significant),*P = <0.05(significant), P=>0.05(N.S=No. significant)**

The liver is the chief organ for metabolism of carbohydrates, lipids, proteins and detoxification of drugs and toxine. However, drugs affect the liver more commonly than any other organ and place the liver at a great risk of toxic damage[13]. After absorption of drugs by the intestine, it reaches the liver via the portal system. Liver disease remains as one of the serious health problems throughout the world[14].

The release of too much thyroxine in the bloodstream is known as throtoxicosis. This may be caused by over activity of the thyroid gland (hyperthyroidism), as in Graves' disease, inflammation of the thyroid or a benign tumour. Thyrotoxicosis can be recognized by a goiter which is a swelling of the neck due to enlargement of the thyroid gland . increased bowel movements , irregular menstrual . cycle rapid or irregular heartbeat , palpitation , tiredness , irritability , tremor . hair loss and retraction of the eyelids resulting in a staring appearance[15]. Normal circulating levels of thyroid hormones are required for both, normal hepatic function and normal bilirubin metabolisum[16].

The diagnosis of thyrotoxicosis is based on clinical symptoms, and characterstic pattern of chances in some serum enzymes such as LDH, ACP, and ALP[17]. LDH is the a cytosolic enzyme mainly present in periportal hepatocytes and released when the cells are lysed by hepatotoxin. The amount of enzyme released is proportional to the extent of damage caused to the cell [18]. Different forms of acid phosphatase are found in different organs such as spleen, kidney, pancreas, liver, bone, and prostate gland [19]. ALP is present in anumber of tissue including liver, bone, intestine, and placenta [20]. The amount of enzymes released depends on the degrees or cellular damage, the intracellular concentration of the enzyme and the mass of affected tissue. The cause of the damage the enzyme released reflects the severity of the damage[21].

In other meaning the elevated levels of the LDH, ACP, and ALP in the rabbit females administered with levothyroxine sodium indicate the hepatocellular damage and alteration in the membrane permeability[22]. Our results are consistent with earlier studies, which strongly suggest that Vitis vinifera L. may protect the structural integrity of hepatocytes and prevent the leakage of cytosolic enzymes into bloodstream[23]. In addition Vitis vinifera L. leaf proved to be effective in reducing the extent of heptocellular damage may be due to the presence of therapeutic phytochemicals such as proanthocynidins and natural polyphenolic. Our finding suggest hyperthyroidism (also known as thyrotoxicosis overactive thyroid) frequently leads to liver dysfunction[24].

Conclusion:

The present study concludes that the aqueous leaf extract of Vitis vinifera L. shows a therapeutic effect against levothyroxine sodium induced thyrotoxicosis in experimental femals of rabbit and levels of LDH, ACP, and ALP are reduced which may be due to presence of bioactive compounds such as flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidine and resveratrol.

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