Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.20, 2016

Aqueous Extract of Grape Leaves Effects on Liver Enzymes and Some Kidney Function Parameters in Hyperthyroidism Female Animals

Muntaha A. Lafta
Department of Chemistry /College of Education Ibn Alhaitham for Pure Sciences/ University of Baghdad

Abstract
Vitis vinifera L. is kind of grape grown widely in Asia, Europe and America.It has many nutritional and pharmacological uses in the Indian traditional medicine, also by European folk healers and many other nations, for treatment of many medical conditions such as nausea , thirst , cough , constipation , smallpox , cholera, hemorrhoids and some of skin and eye diseases.In the present study, *Vitis vinifera L.* leaves was collected from shrub, grown in IRAQ. The aqueous leaves extract used, was prepared and evaluated for kidney functions (urea, creatinine ,uric acid) and liver enzymes activity (AST,ALT) in induced hyperthyroidism female rabbits. Results showed significant decrease ($p<0.05$) in levels and activities of the studied parameters .Our conclusion this herb may inhibits enzymes activity, that was activated by Levothyroxine sodium. So we think there is a relation between the drug and enzymes releasing sources.
Keywords: *Vistis vinifera L.*, L- thyroxin, Urea, Creatinine, Uric acid, AST, ALT.

Introduction

The grape trees usually take the shape of woody vine, using tendrils to climb which are branches that has been modified. Branches can get the length of 56feet (17 meters) if they did not get trimmed[1].

Several preparation from different parts of *Vitis vinifera L.* used in ancient medicine have been derived . Plant leaves, seeds and fruits used in the Indian traditional medicine, also by Ethnopharmacology folk healers and many other nations, for treatment of many medical conditions such as haemostatic, diarrhea, hepatitis, stomachache. Other uses was for blood vessels diseases, atherosclerosis, high blood pressure, many kinds of bleeding, heart attack and stroke and hemorrhoid. Also nausea , thirst , cough , constipation , smallpox , cholera . In addition for some mental disorders like chronic fatigue syndrome and attention deficit-hyperactivity disorder.

Also externally fresh leaves used to lance abscesses, wounds and canker sores healing even some of skin and eye diseases[2].

Antibacterial[3],antioxidant and antidiabetic activities shown by the aqueous extract from leaves of *Vitis vinifera* L.[4], mean while this leaves are rich in procyanidins , anthocyanins flavonoids ,tannins, and also contain lipids ,organic acids, vitamins and enzymes [5,6,7,].

Monagas *et al.* [8]evaluate the chemical compounds found in leaves of*Vitis vinifera* L.by quantitative analysis.

Thyroxine (T_4),and triiodothyronine (T_3) are thyroid hormones produced and secreted by thyroid gland, both play an important role in metabolism regulation. T_4 have a longer half with higher concentration in blood than T3[9]. Triiodothyronine (active form)is produced from its prohormone thyroxin enzymatically by deiodinase. Then thyronamine and iodothyronamine are produced by deiodination and decarboxylation[10].

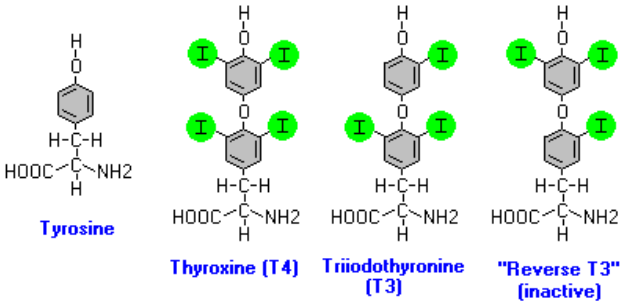

Figure (1) Thyroxine (T4) and Triiodothyronine(T3) structure.

Hypothyroidism is disorder related to a deficiency of thyroxine, which treated by T3 and T4 orally because both of them are well absorbed in the intestine. levothyroxine sodium (T4),is used as therapeutic agent because of its slowly metabolism more than T3,so it is administrated once daily in the form of Levothyroxine which medicaly known as (INN).Some patients can not convert T4 to T3 in this case Levothyroxine is not suitable for treatment.

Excess of thyroxine releasing (i.e. free triiodothyronine, free thyroxin or both of them) can cause

another metabolic disorder that is Hyperthyroidism[11].

Aim of the study

The present study aimed to investigate the effect of *Vitis vinifera L.* , aqueous leaves extract on serum levels of urea, creatinine and uric acid also the activities of AST,ALT enzymes, in induce hyperthyroidism female rabbits.

Methodology

1-Preparation of leaves extract:

Vitis vinifera L. leaves collected from shrub, grown in IRAQ, were prepared and evaluated for the study as follow:

The fresh and healthy leaves washed, cleaned, dried and powdered. distilled water mixed with the leaves powder (25g:250ml),then the suspension was incubated twice ,first at (60C°)for 3hrs then at room temperature for overnight .Later the extract filtered and saved in refrigerator.

Experimental rabbits administrated orally with freshly prepared leaves water extract in dose of (5 ml/ 1.5 – 2.0 kg body weight) .

2-Preparation of thyroxin :

Levothyroxine sodium was supplied by local Pharmacy (Al-Sophee), Baghdad, Al-Adamia, Iraq.

One tablet of drug (Levothyroxine sodium) was freshly prepared and feed to the experimental subjects in equal doses (50µg/kg bodyweight).

3-Experimental

Female ORYCTOLAGUS CUNICULUS rabbits used in this work (weighted 1.5 – 2.0 kg) were purchased from the animal house of city of medicine laboratories / Baghdad(Iraq). Rabbits supplied with healthy food for a time (before the experiment) to maintain their condition, which was vegetable ,protein, bread and multivitamins.

Levothyroxine sodium (50µ/(1.5-2)kg body weight) used to induce the condition of the work (huperthyroiodism) administrated orally ,and it was prepared by dissolving one tablet of Levothyroxine sodium in water freshly.

Four groups each consist of 9 animals were the base of the work as follow:

1- Group one (control)(G1):- Rabbits for two months were orally administered 5 ml of distilled water using feeding solution on daily doses.

2-Group two:- once a day 50 ug of levothyroxine sodium were orally fed to the Rabbits for 1 month.

3-Group three:- for 1 month 50 ug of levothyroxine sodium and 100mg/ml of *Vitis vinifera L.* were treating the Rabbits.

4- Group four:- 100mg/ml of *Vitis vinifera L.* leaves extract were treating Rabbits for two months.

4- Sampling:-

The blood samples were collected using heparinized capillary tube from the hearts of rabbits. 10 minutes of centrifugation at (2000) rpm was used to separate the serum.

5-Lab Work

Plants primary chemical investigation : active chemical constituents such us phenol content, protein, reducing sugar, carbohydrate, alkaloids and tannin were being check for their presence using the tests by series of testes which includes alkaloids [12] , phenols [13], carbohydrates [14], reducing sugar [15], tannin [16] , amino acid and protein [17].

Secondly following parameters studied in this work, were estimated using kits supplied by Biomaghreb company.

1-Urea level determined by enzymatic colorimetric method (Berthelot modified method)[18].

2-Creatinine determined Kinetically without deproteinization[19].

3-Uric acid level determined by enzymatic colorimetric test (uricase-PAP)[20].

4-Estemation of AST,ALT activities was calorimetrically[21].

Statistical analysis

Date presented were the means ± slandered deviations, students, s t-test was used to compare the significance of the difference in the mean values of any two groups ($p \leq 0.05$)was considered statistically significant(100)

The overall predictive values for the results in all studied groups was performed according to program of office Excel 2007

Results and Discussion

Table (1) showed the plants chemical investigation contents in aqueous leave extract of *Visit vinifera L.* grown in IRAQ.

Alkaloids	+
Phenolic content	+
Carbohydrates	+
Reducing sugar	+
Amino acid	+
Proteins	+
Tannins	+

Table(1): Result of plants chemical investigation of aquatic leaves extract of *Vitis vinifera L.*

Table (2) illustrate the levels of urea, creatinine, uric acid, AST and ALT for the control group (G1) and the Levothyroxine sodium induced hyperthyroidism groups (G2,G3 and G4) in the experiment steps

Subjects	No.	Urea Mg/dL Mean±SD	P	Creatinin Mg/dL Mean±SD	P	Uric acid Mg/dL Mean±SD	P	GOT U/I Mean±SD	P	GPT U/I Mg/dL	P
Control (G_1)	9	30±0.43		0.7±0.02		4.6±0.18		6.5±0.07		5.2±0.04	
Levothyroxine sodium treated (G_2)	9	40±1.30	0.001	2.3±0.06	0.001	6.5±0.09	0.001	7.0±0.21	0.001	6.3±0.08	0.0001
Mixed treated (G_3)	9	35±1.6	0.001	1.5±0.06	0.001	5.3±0.10	0.001	6.3±0.10	0.0005	5.5±0.16	0.001
Plant extracted (G_4)	9	29±1.45	0.073	0.9±0.12	0.001	3.0±0.20	0.001	4.2±0.14	0.001	3.5±0.18	0.001

Table (2) Results of the studied parameters in the study

All the results of this work are listed in table (2) and was as follow:

1- All the studied parameters(urea, creatinine, uric acid, AST and ALT) in(G2) showed highly significant compared to control(G1) after orally fed with levothyroxine (INN).

2- The second step which represented with(G3) all the studied parameters showed highly significant compared to control(G1) due to slightly effecting of the treatment by the aqueous leaves extract of *Visit vinifera L.*

3- Group four(G4) showed highly significant in all the parameters except urea which refer to the therapeutic time is not enough.

4- The level of urea and AST,ALT enzymes activities were affected by the extract of *Visit vinifera L.* after duration compared to control which seems that the aqueous extract have an inhibition activity.

antioxidant, anti-inflammatory anticarcinogenic also antimicrobial are some of the biological activities of anthocyanine. coronary heart diseases, blood vessels, blood platelets and lipoproteins have a chance of anthocyanine activities by reducing the risk of cardiovascular diseases [22].

New researches used aqueous leaves extract of *Visit vinifera L.* grown in IRAQ to study therapeutic effect on thyrotoxicosis in induced hyperthyroidism female rabbets .The result was positive and the levels of LDH,ACP and ALP enzymes were reduced[23], and another study on this extract elicited mild and moderates alteration on IgM, IgA and had stimulating effect on IgG[24].

A significant effect on T3, T4 and TSH was offered by *Visit vinifera L.* aqueous leaves extract[25]. Also for the above study condition, the aqueous extract change the levels of LH, FSH, PRO and PRL hormones[26].

In liver function tests we might find few changed such us elevations of the transaminases in case of hyperthyroidism, and liver histology might have some minor changes [27]

Therapy with propyithiouracil may cause an elevation in liver enzymes in hyperthyroidism case[28]. Bombardenei, et.al. [29] showed this percentages 27.4% found with an elevation in AST , and 36.8% in ALT

Protein synthesis with cell growth influenced by thyroid hormones. This accelerating effect on renal development showed by many studies in neonatal rate[30] also shown in this work.

Renal mass (kidney/ body mass) have been affected by thyroid hormones, with an elevation in this ratio due to hyperthyroidism and reducing due to hypothyroidism [31].

Also, similar studies on human and animal showed a reduction in serum creatinine in the controlling of hyperthyroidism ,that is similarly reversible according to[32].

In un treated hyperthyroidism the ratio blood urea nitrogen / creatinine will be high due to the suppression of creatinine concentration with an increase in blood urea nitrogen. Both blood urea nitrogen and creatinine with their ratio will be back to normal levels after treatment. The abnormal elevation in the ratio blood urea nitrogen to creatinine in hyperthyroidism , could be explained by kinetic analysis on urea nitrogen and creatinine, with cardiac output measurement. The results showed a marked elevation in cardiac output, while serum creatinine was reduced because of the increase in renal excretion of creatinine, also due to the reduction in creatinine synthesis. So blood urea nitrogen was high ,because there is an increase in urea nitrogen production at the same time the insufficient excretion of urea nitrogen with the excessive catabolism of protein[33].serum creatinine levels are reduced due to increasing in both glomerular filtration rate and renal plasma flow caused by

hyperthyroidism[34]also a reduction in serum creatinine levels has been stated in sub-clinical hyperthyroidism resulting increasment of serum urea. In contrast, this study showed an increasing in creatinine level. [35].

Thyroid hormones play important role in maintenance of water and electrolyte homeostasis and in the growth and development of the kidney . At the same time, the kidney shared in the metabolism and elimination of thyroid hormones.

It was well known that both hypothyroidism and hyperthyroidism are accompanied with an alterations in electrolytes and water metabolism and in cardiovascular function. In different body parts the electrolyte and water balance are effected by Thyroid hormones

In a not fully understood mechanism, hyperthyroidism increases the kidney to body weight ratio and , in contrast to hypothyroidism which reduces the ratio

Elimination and the regulation of metabolism of thyroid hormones are manipulated by the kidney [36].

Conclusion

The levels of the studied parameters (urea, creatinine ,uric acid ,AST and ALT) were increased due to hyperthyroidism induced by levothyroxine sodium (INN),then lowered after treatment with aqueous leaves extract of *Visit vinifera L.* nearly to the control level or even less. Our conclusion the aqueous leaves extract lowered enzymes activities, so we suppose that there is a relation between the phytochemical content of the aqueous leaves extract of *Visit vinfera L.* and enzymes situation.

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