Grape seed extract role against carbimazole action on thyroid gland and lipid profile in male rats.

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Abstract
The aim of the current study is to identify the role of grape seed extract in thyroid gland and lipid profile disorders induced by Carbimazole 30 mg/kg.b.w. The experience including 40 male rats randomly divided into five groups each group consist of 8 animals, grape seed extract used by dose 150mg/kg daily for the duration of the 30-day study period.

Results showed that the animals treated with carbimazole had a significant decrease(P<0.05) in the concentration of thyroid hormones and increase TSH compared with their normal range level in the control group and the rest of the experimental groups, While groups in which grape seed extract(GSE) interferes with drug showed improvement in thyroid hormones level closer to normal range in control group.

Also the results showed significant increase (P<0.05) in TC, TG HDL, vLDL and significant decrease in LDL in carbimazole group, in addition to damage in thyroid tissue. On the other hand animals treated by both grape seed extract (GSE) and drug showed significant improvement(P<0.05) in TC, TG, HDL, vLDL, LDL and thyroid tissue compared with groups treated with drug only. It was concluded from this study that grape seeds extract had protective role against damage caused by carbimazole.

Keywords: grape seed extract, carbimazole, Thyroid hormones, lipid profile, hypothyroidism.

Physiology Classification Qp-(981)

The research is a part of on MSC. Thesis in the case of third researcher.
دور مستخلص بذور العنب ضد الآثار الجانبية لعقار Carbimazole على الغدة الدرقية ومستوى الدهون

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خلاصة:
أن الهدف من هذه الدراسة هو التحقق من دور مستخلص بذور العنب في تقليل الآثار الجانبية لعقار Carbimazole على الغدة الدرقية ومستوى الدهون في الجسم، وقد تم استخدام العقار بجرعة 30 ملغم/كغم من وزن الجسم أما مستخلص بذور العنب فقد تم استخدامه بجرعة 150 ملغم/كغم من وزن الجسم لمدة 30 يوم، وتتم استخدام 40 جرذ من الذكور البالغة حيث قسمت إلى خمس مجموعات وكل مجموعة تحتوي على ثمانية حيوانات. اظهرت النتائج ان الحيوانات المعالمة بعقار Carbimazole اظهرت انخفاض معنوي (P<0.05) في تركيز هرمونات الغدة الدرقية وارتفاع معنوي (P<0.05) في تركيز هرمونات السكر في الدم، بالإضافة إلى ان مستخلص بذور العنب كписан في تركيز الهرمونات ونسبة الصفرة، كذلك اظهرت كمبيت المعالمة بالعقار ارتفاع معنوي (P<0.05) في تركيز TC,TG,HDL,vLDL، وانخفاض معنوي (P<0.05) في تركيز LDL. باللوم مع السكر في الجسم، أصبحت المجموعة المعالمة بالعقار مع مستخلص بذور العنب تحسن في تركيز الهرمونات والدهون، حيث قسمت إلى خمسة مجموعات. هذه المجموعة المعالمة بالعقار فقط، تستنتج من هذه الدراسة أن مستخلص بذور العنب دفع في حماية الغدة الدرقية وتحسين مستوى الدهون في الجسم ضد الاضرار الناجمة عن الآثار الجانبية لعقار Carbimazole.

الكلمات المفتاحية: مستخلص بذور العنب، الكاربيمازول، هرمونات الغدة الدرقية، نسبة الدهون، حسا/البحث جزء من رسالة الماجستير للباحث الثالث.
**Introduction**

Thyroid gland common largest endocrine gland in the body, its shape resembles butterfly on both sides of the trachea. Thyroid hormones plays an important role in regulation metabolism in all cells of the body, Thyroid hormone T4 (L-3,5,3,5-tetraiodothyronine and T3 (L-3,5,3-triiodothyronine) responsible for regulation of Proteins, fats and carbohydrates metabolism(1), and doing its functioning on the cells by thyroid stimulating hormone receptor TSHR that find on the cells DNA(2). Any disorders occurs in thyroid hormones (hypo- hyperthyroidism ) led to disorders in the cells function of the body(3).

Carbimazole and L-Thyroxin known as drugs to treat thyroid disorders it is also experimentally used to induce hypothyroidism and hyperthyroidism in laboratory animals.(4) These drugs have an effect on oxidative stress and effect on lipid profile (TC, TG, HDL, LDL, vLDL)(5,6). In addition it may be cause damages in the tissue of the thyroid gland(4,7). Grape seed extract GSE has a major role in free radicals resistance that led to oxidative stress, as well as protecting cells from the toxic effects of drugs (8).

**Materials and Methods**

Experience design: In this study 40 male rats 200-250g weight, 3-4 months age divided into five groups each group consisted of 8 rats, (C) group considered as control given 1ml of distil water for 45 day, (T1) first group given orally dose of carbimazole 30mg/kg daily for 45 day to induce hypothyroidism, (T2) second group given orally dose of GSE 150mg/kg daily, (T3) third group given orally dose of carbimazole 30mg/kg for fifteen days then given oral dose of carbimazole 30mg/kg and GSE 150 mg/kg daily for 30 day, (T4) fourth group given oral dose of carbimazole 30 mg/kg for fifteen days then given oral dose L-Thyroxine 20mg/kg to the end of the experiment for the purpose of comparing the ability of the grape seed extract and the drug to resist the negative effects of carbimazole.

**Chemicals:** Used carbimazole drug to induce hypothyroidism dose 30 mg/kg.B.W it is a common drug for the group of hyperthyroidism.

**Plant extract:** In the present study local Iraqi Grape fruits purchased from local Iraqi markets, Seeds were isolated and dried in the shade, then grinded into an electric mixer to get a powder for use in the soxhlet apparatus for a water extract of grape seeds.

**Determination of some biochemical parameters**

Determination of lipid profile (TC, TG, HDL, LDL, vLDL) by kits provided from BioMerieux France company. determination of (TSH, T4, T3) by kits BioMerieux France company.

**Histological study:** Histological sections of thyroid gland was prepared according to the method(9).

**Statistical Analysis:** All results under study were subjected for statistical analysis in order to know the significant differences between the control group and other groups in both groups by using F-test at 0.05 probability level(10).

**Results and Discussion:**

**Effect of carbimazole and GSE in (TSH, T4, T3).**

Notes through the results table(1) that the animals treated with carbimazole in the first and the third groups had a significant decrease(P<0.05) in the concentration of
thyroid hormones (T3, T4) compared with their normal range level in the control group and the rest of the experimental groups, that maybe due to known mechanism of carbimazole to inhibit peroxidase enzyme TPO action which it an important enzyme in the synthesis of thyroid hormones (11,12).

Third group in which grape seed extract(GSE) interferes with drug, so that maybe explain the reason that T4 hormone level in this group closer to normal range than its level in the first group maybe due to GSE flavonoids, Flavonoids have the ability to inhibit the action of andeiodinase D1 enzyme that converts T4 to T3 in liver this arise the level of T4 (13).

What mention above may explain that there is an increase in the level of the TSH hormone reached to the significant degree(P<0.05) in the first group compared with the control, while it did not in the third group. Because it is known that there is an inverse relationship between the level of hormone TSH and the level of thyroid hormones according to the negative feedback mechanism (14).

**Table(1): Effect of grape seed extract and carbimazole in TSH, T3, T4 level.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>T3 nmol/l</th>
<th>T4 nmol/l</th>
<th>TSH µIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
<td>2.43±0.11</td>
<td>83.66±1.6</td>
<td>0.163±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>0</td>
<td>b</td>
</tr>
<tr>
<td>T1</td>
<td>1.86±0.07</td>
<td>58.00±1.9</td>
<td>0.250±0.014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bc</td>
<td></td>
<td>2</td>
<td>a</td>
</tr>
<tr>
<td>T2</td>
<td>2.16±0.27</td>
<td>82.66±4.24</td>
<td>0.165±0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ab</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>T3</td>
<td>1.56±0.07</td>
<td>66.66±4.34</td>
<td>0.180±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c</td>
<td></td>
<td>b</td>
<td>ab</td>
</tr>
<tr>
<td>T4</td>
<td>2.36±0.01</td>
<td>82.20±3.49</td>
<td>0.167±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>LSD</td>
<td>0.34</td>
<td>10.9</td>
<td>0.076</td>
<td></td>
</tr>
</tbody>
</table>

**Effect of carbimazole and GSE in lipid profile.**

In table(2) and table(3) results showed significant increase(P<0.05) in TC, TG, HDL, vLDL parameters in carbimazole group compared with control and GSE group also the results showed significant decrease(P<0.05) in LDL concentration in carbimazole group compared with control.

On the other hand in GSE group result showed that there is significant decrease(P<0.05) in all studied lipid parameters except LDL results which was close to control, while animals treated with carbimazole and GSE in third group showed significant decrease(P<0.05) in TC, TG, HDL, vLDL and significant increase (P<0.05) in LDL compared with first group which treated with carbimazole only.
As we explained Carbimazole action cause decrease in thyroid hormones, and these hormones can regulate fat metabolism and affect level of lipids. So decrease thyroid hormone lead to high cholesterol because of its control in cholesterol synthesis through gene expression and mRNA reproduction(2). Then deficiency of thyroid hormones leads to imbalance in fat digestion, absorption, synthesis or prevent activation of their receptors(13, 15). Hypothyroidism causes decrease activity of Lipoprotein lipase in fatty tissues and liver cells which is responsible for fat metabolism and this leads to a high concentration of TG, HDL, vLDL(5), hormones is require to regulate LDL. Low level of LDL in first group maybe due to lack of thyroid hormone, because of enough concentration of this receptors and activate cholesterol ester transfer protein which is responsible for transfer cholesterol ester from HDL to LDL led to LDL synthesis (16). Also the LDL increasing in maybe due to flavonoids anti-oxidant, inflammatory properties to protect LDL from oxidation by free radicals(17,18).

Conversely was there an increase in LDL level in GSE group close to normal range in control as well as in third group compared with control and third group, anti oxidants compounds in grape perhaps inhibit LDL oxidation and convert it to bad form to be able to inter inflammatory cells in blood vessels walls as this way we can found high
level of LDL in serum but not into bad form yet, according to fact that LDL molecules must be oxidized to be able to enter inflammatory cells (19).

Concerning the significant decrease in TC, TG, HDL, vLDL in GSE group compared with control and in group treated with carbimazole and GSE compared with first group, proanthocyanidins one of grape compounds (20) had role in reducing lipid concentration by promote the transfer of cholesterol and increase the effectiveness of its secretion by bile and (21). Polyphenolic compounds in GSE like gallic acid, catechin, epicatechinhave had the ability to reduce cholesterol by inhibition of the pancreatic cholesterol esterase enzyme which is hydrolyzes cholesterol in food and facilitate the solubility in the colloidal micelles to facilitate absorption and that led to reduce the absorption of cholesterol to blood stream in addition to the activity of these compounds to stimulating the synthesis of bile acid from cholesterol (22).

**Histopathological study**

Histological sections of thyroid gland in control (Fig.1) showed natural follicles filled with thyroglobulin and the follicles are padded with one cells line and normal number of c-cells among the follicles. While animals treated with carbimazole only (Fig.2) showed marked necrosis in the cells lining the thyroid follicle and presence of small amounts of thyroglobulin Interspersed with large gabs and there are few numbers of c-cells. On the other hand thyroid tissue in GSE group (Fig.3) was contain medium follicles filled with thyroglobulin, natural follicular cells, many numbers of C-cells.

Carbimazole and GSE group histological examination (Fig.4) showed a marked improvement in thyroid tissue compared with first group, various follicles in different size filled with thyroglobulin and follicles lined with natural cubic epithelial cells and composed of one row of cells as well as many of C-Cells. group with carbimazole+ L-Thyroxine showed The follicles appear to contain bubbles(V) and clear hyperplasia of follicular cells It was observed that protrusions (papillae) extending into the lining of the follicles.

It has been shown through the histological examination that there are increasing damage of thyroid tissue in the animals that were treated with the drug Carbimazole, it was more severity in first group which treated with Carbimazole only, because of this drug has toxic effects on the thyroid tissue and causes sever damage (7), and it causes reduction in thyroid hormones and that causes a rise in the concentration of TSH, and TSH continuous stimulation of the tissues, the tissue is exposed to hyperplasia and to pathological changes (23).

While in groups that treated with grape seed extract we can found marked improvement in thyroid tissue specially in third group, that maybe consider as evidence that grape seeds compounds had the ability to resist Carbimazole negative effects, Because of grape seed extract protects cells from oxidative stress caused by this drug which causes damage and cell death (24). Also polyphenol and other compound in GSEP protects cells and blood vessels against damage caused by drugs and Prevents damaged by inhibiting the action of enzymes that lead to cell dissolution (25). Flavinoids one of the most common compounds in grape seed
extract have been shown to play a role in stimulating TSH receptors in the thyroid gland to increase C-cells proliferation (13).

When we compare the histological examination results of GSE+ Carbimazole and results of L-thyroxine+ Carbimazole we can observe a clear superiority in favor of the GSE+ Carbimazole group. Maybe that means the animals with L-thyroxine in forth group did not prevent the negative tissue effects of Carbimazole. L-thyroxine drug as alternative hormone when inter the body converted into T3 hormone in tissues this a rise thyroid hormones(3), and that led to decrease TSH concentration because of the inverse relationship between thyroid hormones and TSH (14), at this way thyroxine doses may reduce the concentration of TSH and this results in a small number of C-Cells (26). Also it causes a difference in the thickness of epithelial cells of follicles(4).

Fig.1: thyroid tissue in control, follicles filled with thyroglobulin (T), C-Cells (C), padded cells line (E) 40X

Fig.2: Thyroid tissue in carbimazole group, thyroglobulin (T), large vacuoles (V), necrosis in follicular cells (E). 40X

Fig.3: Thyroid tissue in GSE group, Medium follicles filled with thyroglobulin(T), follicular cells(E), C-cells (C).

Fig.4: Thyroid tissue in carbimazole+ GSE, filled with thyroglobulin(T), follicular cells (E), many numbers of C-Cells(C).
Fig. 5: Thyroid tissue in carbimazole+L-thyroxine group. Medium follicles filled with thyroglobulin (T), hyperplasia protrusions (P), C- cells (C).

References


