

# Effects of Iraqi Propolis, Carbimazole and Levothyroxine on the Liver: Histopathological Study in Normal Female Rats

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## Abstract

**Background:** Liver has an essential role in the metabolism of thyroid hormones. Disorders of thyroid hormones level causes' abnormal liver function, so the liver function and thyroid hormones level affects each other. This study aim to investigate the effect of propolis (PR), carbimazole (CB), levothyroxine (TR) and their PR combinations on the liver biochemical and histopathological parameters in normal adult rats.

**Methods:** Forty-two adult female albino rats randomized into six groups: control, PR, CB, TR, PR + CB and PR +TR. Rats orally treated by gavage for six weeks. Liver biochemical and histopathological analyses performed.

**Conclusion:** TR in a dose of 0.1 µg/g daily for six weeks produced a significant elevation ( $p < 0.001$ ) in serum AST and ALT enzymes level compared to control in normal rats. CB dose of 0.01 mg/g displayed a significant increase in serum AST ( $p < 0.005$ ) and ALT ( $p < 0.05$ ) enzymes level compared to control. PR dose of 50 mg/kg produced a non - significant difference in liver enzymes level compared to control. Moreover, the rats treated with PR+TR or PR +CB combinations show a significant decrease in serum AST and ALT enzymes level compared to TR or CB alone groups. These results confirmed with a histopathological results to indicate a hepatoprotective effect of propolis.

**Key words:** Iraqi propolis, Carbimazole, Levothyroxine, Liver, AST, ALT

## Introduction

Liver has an essential role in the metabolism of thyroid hormones because the liver is the place for the synthesis of proteins essential for binding of thyroid hormones. In addition, liver is a major site for metabolism of thyroid hormones. Furthermore, thyroid hormones level affects liver function, disorders of thyroid hormones level causes' abnormal liver function, so the liver function and thyroid hormones level affects each other<sup>1</sup>. Liver dysfunction can be observed as a complication of hyperthyroidism or after the use of thionamide antithyroid drugs, these drugs

act by the blockage of iodine-tyrosine coupling and iodine organification causes inhibition of biosynthesis and production of thyroid hormones from the thyroid gland. All of antithyroid drugs including carbimazole prodrug and its metabolite methimazole are carrying a risk of causing liver dysfunction and their toxicity in over 40 years age people is more common than others<sup>2</sup>. Carbimazole and its metabolite methimazole reported to cause hepatotoxicity<sup>3-5</sup> and increases the risk of hepatitis<sup>6,7</sup>.

Levothyroxine (TR) is a synthesized thyroid hormone applied in the treatment of hypothyroidism, causes upregulation of thyroid stimulating hormone receptors causes increase in the secretion of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>). Furthermore, TR exerts effects on the DNA transcription causes an increase in serum protein level<sup>8</sup>. Hypothyroidism can affect liver, causes decrease in hepatic perfusion and cholestatic

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hepatitis<sup>9</sup>. Previous case report studies revealed that treatment with TR causes hepatic problems<sup>10-13</sup>. Another study revealed that T<sub>3</sub> causes stimulation of the proliferation of hepatocytes<sup>14</sup>. Further study on thyroid hormone of more than physiological levels revealed no clinical effects on liver injury markers found after two weeks treatment<sup>15</sup>.

The resinous substance called propolis collected by bees from plants exudates and the bees used it to cover holes in the honeycombs. Propolis is rich in various biochemical contents, including flavonoids, polyphenols, phenolic acids, esters, aldehydes and phenolic ketones, amino acids, terpenes, sterols, vitamins and others<sup>16</sup>. It was reported that Iraqi propolis have a protective effects on hepatocytes carbon tetrachloride hepatotoxicity<sup>17</sup>. In addition, a study on Nigerian propolis revealed that propolis exerts a protective effect on liver and pancreas against oxidative stress induced by hyperglycemia in adult rats<sup>18</sup>. The constituents of Iraqi propolis studied, and its main constituents includes polyphenolics, like flavones, flavonones, terpenes, fatty acid, phenolic acid and esters. Furthermore, about 32 different flavonoids detected in Iraqi propolis extracts using HPLC-ESI-MS<sup>19</sup>.

This study aim to investigate the effect of PR, CB, LR and their PR combinations on the liver biochemical and histopathological parameters in normal adult rats. Also, to investigate the effect of CB and LR combinations with Iraqi PR on the liver functions in normal adult rats.

## Materials and Methods

### Materials

Iraqi PR sample collected from the town called Al-Tarmiyah located northeast in about 60 km from Baghdad, Iraq, in various seasons through rasping it off from surrounds of hives of bees and kept at 4°C. Dimethyl sulfoxide obtained from sigma. Film coated tablet of carbimazole 5mg obtained from Remedica Ltd. pharmaceutical Company in Limassol-cyprus- Europe. Levothyroxine (Euthyrox 100 ug) obtained from Merck KGaA Research and product development in Darmstadt, Germany.

### Methods

After adaptation period, forty- two adult Sprague

female rats (from the drug control and scientific research department in Baghdad) (weigh up 210–300 mg) included in this study. The animals positioned in plastic cages in pairs, in the normal conditions in the lab with regard to air, humidity; the temperature was  $28 \pm 3^\circ\text{C}$ , with 12 hours light, and 12 hours dark cycle, provided water ad libitum. Rats divided haphazardly into six groups with seven rats each. The first Group considered as the Control, received 0.5 ml of normal saline once daily for two weeks. Group 2, PR group, received Iraqi PR 50 mg/kg b.w. dissolved in normal saline once daily for six weeks. Group 3, CB group, received CB 0.01 mg/g b.w. dissolved in normal saline once daily for six weeks. Group 4, TR group, received TR 0.1  $\mu\text{g/g}$  b.w. dissolved in normal saline once daily for six weeks. Group 5, PR + CB group, received PR 50 mg/kg b.w. and CB 0.01 mg/g b.w. dissolved in normal saline once daily for six weeks. Group 6, PR + TR group, received PR 50 mg/kg b.w. and CB 0.01  $\mu\text{g/g}$  b.w. dissolved in normal saline once daily for six weeks. The weight of rats measured at the beginning of the experiment and then measured every two weeks.

After six weeks period of treatments, the rats were killed and then the blood samples were taken by intracardiac puncture and the serum collected by centrifugation of the blood samples for biochemical tests. The liver excised for fixation and used in histopathological examination.

### Biochemical Analysis

Serum levels of aminotransferases (AST) and ALT) measured using Cobas c111.

### Histopathological investigation

Histopathological investigation was prepared by fixing the tissues in 10% solution of formalin for about 4 days, after that the tissues prepared as sections, and covered by paraffin. The histological pieces cut in size of 4–5  $\mu\text{m}$  and stained using routine stain hematoxylin (H) and eosin (E). The stained sections examined by a histopathologist for the damage of tissue and epithelium disruption or infiltration under a light dissection microscope.

### Statistical Analysis

Statistics achieved using software called Graph Pad

Prism (version 7.0, Inc., San Diego, CA). Descriptive statistics data presented as mean  $\pm$  SEM for all estimated parameters. Statistical comparisons between the groups performed with one-way analysis of variance (ANOVA), with post-test called Tuckey's Multiple Comparison test. All p values  $<0.05$  considered significantly different.

## Results and Discussion

### Effect of different treatments on liver biochemical tests in female rat:

The results of this study revealed that administration of TR in a dose of 0.1  $\mu\text{g/g}$  daily for six weeks produced an elevation ( $p < 0.001$ ) of AST and ALT enzymes level in serum compared to control (figure 1). These results of TR effects are consistent with case report studies<sup>10-13</sup>, which revealed that treatment with TR causes hepatic problems. However, these results are not in cope with a previous study on thyroid hormone of more than physiological levels that revealed no clinical effects on liver damage markers found after two weeks of treatment, the duration of treatment in this study is different, it was 6 weeks period<sup>15</sup>.

Furthermore, the rats that received 0.01 mg/g CB displayed a significant increase of AST ( $p < 0.005$ ) and ALT ( $p < 0.05$ ) enzymes level in serum compared to control (figure 1). These results of CB effects are consistent with previous studies revealed that CB and its metabolite methmazole reported to cause hepatotoxicity<sup>3-7</sup>.

The results of this study discovered non - significant difference in liver enzymes level after administration of 50 mg/kg PR compared to control. Moreover, the rats treated with PR and TR combination show a significant decrease of AST ( $p < 0.001$ ) and ALT ( $p < 0.05$ ) enzymes level in serum compared to TR alone group. In addition, the rats treated with PR and CB combination show a significant decrease of AST ( $p < 0.001$ ) and ALT ( $p < 0.05$ ) enzymes level in serum compared to CB alone group (figure 1). These results confirmed that propolis have a hepatoprotective effect on the liver against TR and CB induced hepatotoxicity, this hepatoprotective effect of PR may related to its antioxidant activity. The

results of PR effects on the liver are consistent with previous studies on propolis protective effects on the liver<sup>16-18</sup>.

Histopathological study of Liver function in adult female rats exposed to various treatments:

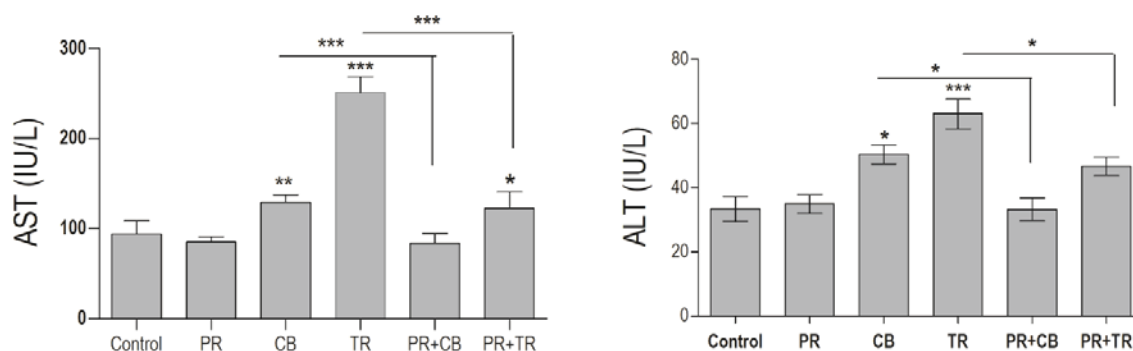
The liver tissue investigation of the control group displayed normal histological construction of the liver. Hepatic lobules that consisted of hepatocytes organized in hepatic cords radiating to the periphery of the lobule from the central vein. The sinusoids separate the cellular cords, as shown in (figure 2a).

The section of liver from female rat treated with 50mg/kg body weight of propolis revealed a significant improve compared with other groups. Therefore, clear no changes notice shows normal histological structure such as hepatocytes, lobules of liver and cellular cords separated by sinusoids (figure 2b).

Liver of rats treated with CB (0.01 mg/g) revealed narrow central veins with congestion and irregular arrangement of hepatocyte with pyknotic nuclei and liver sections, in addition to enlarged sinusoid spaces (figure 2c). While the liver of rats treated with PR + CB showed the effect of moderate central vein narrow with normal hepatocytes compared with control non-treated group (figure 2f). These histopathological results are consistent with the biochemical tests in this study, indicated the hepatoprotection effect of PR against CB induced hepatotoxicity.

The liver of rats treated with TR (0.1  $\mu\text{g/g}$ ) revealed damages included clear enlarged central veins and cytoplasmic vacuolization (figure 2d). While the liver section of female rat treated with TR + PR displayed normal lobular construction with central vein and radiating hepatic cords compared with plate treated with CB or TR alone (figure 2e). These histopathological results are consistent with the biochemical tests in this study, indicated the hepatoprotection effect of PR against TR induced hepatotoxicity.

To the best of our information, this is an original study about the comparisons and combinations of PR with CB or TR effect on the liver.



**Figure 1:** Changes in A (AST) and B (ALT) levels after exposure to PR (propolis), CB (carbimazole), TR (levothyroxine), PR+CB and PR +TR treatments. Values are stated as mean  $\pm$  SEM (\*\* $p < 0.005$ , \*\*\* $p < 0.001$ , \* $p < 0.05$ ).

**Figure 2:** Light micrograph for liver (stained with H and C) 20X. (a) Control, shows normal central vein (black arrow) and hepatocytes (thin arrow) arranged in irradiation manner. Normal sinusoid space (head arrow), (b) Propolis (PR) (50 mg/kg) shows normal central vein (star), hepatocytes (black arrow) and normal sinusoid spaces (white arrow). (c) Carbimazole (CB) (0.01 mg/g) revealed narrow central veins with congestion (red star), pyknotic nuclei (head arrow), and enlarged sinusoid spaces (red arrow). (d) Thyroxine (TR) (0.1  $\mu$ g/g) revealed enlarged central veins (white star), pyknotic nuclei (white arrow), enlarged sinusoid spaces (red arrow). (e) PR + CB treatment showing narrow central vein (star) and normal hepatocyte (thin arrow). (f) PR + TR treatment show normal histological structure of central vein (thick arrow) and surrounding hepatocytes (thin arrow).

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**Conflicts of Interest:** The authors state that they have no conflicts of interest.

**Ethical statement:** All tests performed in agreement with the National Institute of Health Guidelines for the Treatment and Use of Laboratory Animals (86/609/EEC) and permitted by Basrah University, College of Pharmacy Ethical Committee.

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