Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Role of xylo-oligosaccharides in relieving complications accompanied to carbimazole drug administrated with 1% saline in female Wistar rats

Doaa S. Foda[1*] and Shaimaa A. Nour[2]

Abstract

Background Ameliorating the complications of pharmaceutical drugs by natural compounds or probiotics is now a medical strategy. The anti-thyroid drug carbimazole was reported to cause some complications as liver and kidneys dysfunction besides the harmful effect on the structure of the thyroid gland in addition to weight gain during thyroid treatment. The aim of the present work was suggesting xylo-oligosaccharides extracted from *Aspergillus terreus* xylanase degradation of xylan for the first time as a candidate for ameliorating some of the drug-associated complications.

Results The present work reported that the administration of carbimazole drug only to female Wistar rats for three weeks leads to significant decrease in serum levels of ALT, AST and urea ($p \leq 0.05$) which reflected a status of a lazy liver. On the other hand, there was a significant increase in serum levels of total protein content, creatinine and calcium ($p \leq 0.05$). A detected non-significant decrease in serum T4 accompanied with significant increased levels of T3 ($p \leq 0.05$) and a normal serum TSH were observed in this group compared to the control group. Histological examinations on liver, kidneys and thyroid tissues revealed the effect of the drug on their cells shape which reflected the malfunction of these organs. Co-administration of xylo-oligosaccarides to carbimazole in rats significantly improved most of the changed serum parameters levels in addition to a marked modulation in the histological examination of both the liver and kidney tissues. More histological modulations were displayed in the thyroid tissues than those observed in liver and kidneys tissues.

Conclusion Accordingly, it can be concluded that xylo-oligosaccarides extracted from *Aspergillus terreus* xylanase degradation of xylan are suggested to be a safe therapy in ameliorating carbimazole drug-associated complications.

Keywords Carbimazole-*Aspergillus terreus* xylanase-Xylo-oligosaccharides, Body organs structure and functions

1 Background

Hyperthyrodism is a disease characterized by the over expression of the thyroid hormones T4 &T3 (thyroxine & tri-iodothyronine) accompanied to a decrease in TSH (thyroid-stimulating hormone) in the bloodstream [1]. Thionamides, such as carbimazole (CBZ) drug, are anti-thyroid medications used for treatment of the case. CBZ is a 3-carbethoxy methimazole, metabolized to methimazole (MMZ) in the liver. MMZ represents the active

*Correspondence:
Doaa S. Foda
d.foda2018@gmail.com
[1] Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, El Buhouth St., Dokki, Giza, Egypt
[2] Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, El Buhouth St., Dokki, Giza, Egypt

compound of the drug that acts finally on the thyroid gland and causes a hypothyroid case [2–6].

Knudsen et al. [7] reported that slightly differences in the thyroid functions can affect the body mass index and cause obesity. Body weight gain is one of the main side effects that exists during the treatment of hyperthyroidism and reaching a hypothyroid case. Gradual increase in body weights is associated to thionamides therapy in hyperthyroid human cases [8–10].

This gain in body weights can be attributed to the altered metabolism in the body cells due to the changed and decreased thyroid hormones levels. This case can be accompanied to the change in appetite and energy homeostasis [8]. Another important side effects are also observed during hyperthyrodism treatment by CBZ/MMZ such as liver and kidneys dysfunction due to tissue organs necrosis and degeneration [11–14]. A recent study denoted that carbimazole drug affected the lipogenic gene expression in liver cells of hyperthyroid patients leading to abnormal lipid profile [15].

Many studies were performed to inspect the effect of CBZ/MMZ on the thyroid gland structure. Ahmed et al. [16] studied the effect of MMZ administrated to rats for a month on the thyroid structure recording many histological distortions in the thyrocytes. Recently, Hossain et al. [17] denoted many histochemical and histological changes in the thyroid gland of rabbits treated with carbimazole for a period of a month.

Xylo-oligosaccharides (XOS) are oligosaccharides formed by the linking from 2 to 7 xylose molecules by β-1,4-glycosidic bonds. They are obtained from the byproducts of grains. Researchers reported that XOS exert many beneficial biological activities such as antioxidant, anti-inflammatory and antimicrobial properties besides its wide range in preventing diseases mainly cardiovasucular and endocrine ones [18].

The aim of our study was to explore the effect of XOS extracted from *Aspergillus terreus* xylanase degradation of xylan [19] as a co-administrator to carbimazole drug to ameliorate the drug's associated complications in female Wistar rats.

2 Methods

2.1 Extraction and preparation of XOS

2.1.1 Production of Aspergillus terreus xylanase

In the current study, XOS was prepared by the enzymatic hydrolysis of beech wood xylan polymer (Sigma, USA) using xylanase prepared in our laboratory. The used xylanase in the current study was prepared according to Nour et al. [19], in which *Ricinus communis* waste was fermented under solid-state fermentation using *Aspergillus terreus* RGS Eg-NRC (accession number MW282328). In brief, (1 g/flask) of *Ricinus communis*, moistening agent (3 ml), corn steep (1%), KH_2PO_4 (6.5 g/l), glucose (4%), inoculum (3 ml), and incubation period at 30 °C for 11 days.

After incubation on an orbital shaker (150 rpm) for 1 h, the fermented substrate was extracted with 50 ml distilled water and centrifuged at 5000 rpm at 4 °C for 10 min. The enzyme analysis was performed on the generated supernatant [19].

2.1.2 Enzyme, protein estimation and purification of xylanase

Xylanase activity was assayed according to Bailey et al. [20] by incubating 0.5 ml of diluted enzyme extract and 0.5 ml of 1% (w/v) xylan from beech wood (Sigma, USA) solution in 50 mM sodium phosphate buffer pH 5.8 at 50 °C for 30 min; the enzyme reaction was stopped by adding 3.0 ml of DNSA (3,5-dinitrosalicylic acid was obtained from Pan Reac, Barcelona, Spain) reagent and boiling for 5 min. After cooling, the xylose sugar emitted was detected at 540 nm. One unit of xylanase activity (U) is defined as the quantity of xylanase that releases 1 mol of xylose per mL of crude enzyme extract per minute. Protein content was determined according to Lowry et al. [21]. Bovine serum albumin was used as a standard and followed by enzyme purification by fractional precipitation using ammonium sulfate. The fraction 60–70% ammonium sulfate is used in the hydrolysis of xylan.

2.1.3 Hydrolytic conditions for xylo-oligosaccharides production

The ability of the produced enzyme to hydrolyze beech wood xylan polymer was performed in 250-ml screw-caped bottles in which 100 ml of 2% xylan (in 50 mmol/l phosphate buffer pH 5.8, enzyme solution (5 U/ml)) was carried out at 40 °C for 3 h of incubation. The reactions were stopped by boiling for 10 min to denaturation of the added enzyme followed by centrifugation for 10 min at 7000 rpm (4 °C) and then air drying of the clear supernatant. The amounts of reducing sugar of hydrolysis products were detected using DNSA methods. Hence, the hydrolysis product patterns were determined by thin-layer chromatography (solvent system and spraying agent as in Adachi et al. [22]).

2.2 In vivo research

2.2.1 Animals

Healthy female Wistar albino rats weighing between 100–130 g were bought from the animal house's breeding unit at National Research Centre, Giza, Egypt. The animals were housed at controlled temperature and humidity levels and fed a standard pellet rodent diet and unlimited

access of water. Animals were weighed weekly till the end of the experiment. The experiment was carried out under the granted permission of the medical ethical committee of the National Research Centre (No. 18157).

2.2.2 Drug dose preparation

Carbimazole tablets (5 mg) was purchased from the pharmacy and dissolved in 1% saline. The dose of the drug (83 mg/kg b.w) was prepared equivalent to the amount of the drug that contains 0.02% methimazole. The drug dissolved in saline was added to drinking water for rats.

2.2.3 Experimental design

The animals were divided into four groups (6 animals for each group).

Group 1: Rats were given orally carbimazole (83 mg/kg b.w) dissolved in 1% saline in drinking water for three weeks.

Group 2: Rats were given orally carbimazole (83mg/kg b.w) dissolved in 1% saline and xylo-oligosaccharide (0.12 g/kg) [23] in drinking water for three weeks.

Group 3: Rats were administrated 1% saline in drinking water for three weeks.

Group 4: Rats served as normal control with no supplementations.

2.2.4 Preparation of serum

During the duration of the experiment, the body weights were recorded every week. At the end of treatment period, blood samples were drawn from the animals via puncturing the retro-orbital venous plexus with a thin sterilized capillary tube then the rats were dissected by dislocation. Blood specimens were centrifuged at 4000 rpm for 10 min for serum preparation. Serum was then separated and was stored at − 20 °C for biochemical analysis.

2.2.5 Biochemical studies

Biochemical serum parameters were determined as reported by Foda et al. [24].Total serum calcium was calorimetrically determined according to Gindler and King [25]. Thyroid hormones were detected as described by the manufacture kit.

2.2.6 Histological examinations

Tissues samples were fixed in neutral 10% buffered formalin at room temperature. After fixation, tissues were dehydrated through graded alcohol solutions and embedded in paraffin. Sections (4 µm thickness) were stained with hematoxylin and eosin and examined under a light microscope for histopathological analysis [26].

2.2.7 Statistical analysis

Results were presented as mean data ± S.D of the different groups. One-way ANOVA test was run by using SPSS (Statistical Package for the Social Sciences) program (version 7.5) for checking the significance between groups. A statistical significance difference was detected at $p \leq 0.05$.

3 Results

3.1 Xylo-oligosaccharides extraction results

The hydrolysis of xylan from beech wood by *Aspergillus terreus* xylanase was done after 3 h. The amount of reducing sugars released was 15.11 mg/ml. In addition, various sizes of oligosaccharides were detected by thin-layer chromatography (Fig. 1) and these results agreed with our previous paper [19].

3.2 Biochemical results

3.2.1 Effect on body weight

The effect of the administration of carbimazole drug only and xylo-oligosaccarides co-administration for three weeks on the body weight of rats was shown in Table 1. Significant increasing levels represented by (28, 26.3 and 26.04%) were found in carbimazole administrated group, the co-administrated group and the normal control group, respectively, with respect to their corresponding initial weights.

No increase in body weights but a slight non-significant decrease (− 4%) was observed in the control group

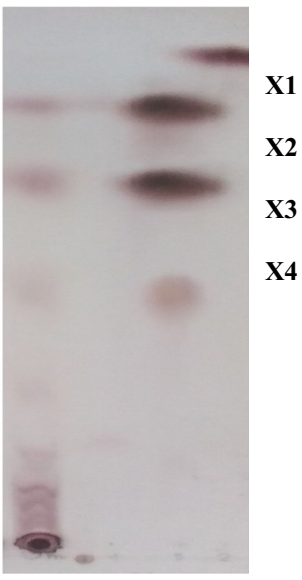

Fig. 1 TLC plate of hydrolysis product of xylan by *Aspergillus terrus* xylanase at 3 h. X1: mono, X2: Di, X3: Tri, X4: Tetra, lane 1: XOS: (xylo-oligosaccharides). TLC: Thin-layer chromatography

Table 1 Effect of carbimazole drug and xylo-oligosaccharides co-administration on body weight of female rats

Parameters	Groups			
	Carbimazole (positive)	Carbimazole& and xylo-oligosaccarides co-administration (Treated)	1% saline control	Normal control
Initial bd.wt	97.8 ± 10.6&	99 ± 9.77&	133 ± 12.13	124.25 ± 5.80
Final bd.wt after 3 weeks	137.43 ± 12.37*,&	134.5 ± 14.07*,&	128 ± 9.65&	168 ± 7.04*
% Change compared to initial bd.wt	+ 28	+ 26.3	− 4	+ 26.04

Data represented as mean ± S.D. *P* significant at $p \leq 0.05$

P^* significant to corresponding initial bd.wt while $p^{\&}$ significant to the normal control gp

administrated 1% saline compared to its corresponding initial body weight during the period of the three weeks.

3.2.2 Effect on serum liver and kidney functions

Serum liver and kidney functions of carbimazole drug administration and the co-administration of carbimazole and xylo-oligosaccarides for female rats were demonstrated in Table 2. There was a significant marked increase in serum total proteins, total calcium and creatinine levels and a marked significant decrease in ALT, AST and urea serum levels in the group of rats administrated carbimazole only (positive group) with respect to the normal control group.

Compared to the carbimazole (positive) group, decreasing significant levels of serum total protein and creatinine were observed in the group administrated carbimazole and xylo-oligosaccarides (treated group). Also a marked significant promotion was observed in serum levels of AST.

Non-significant recovery was observed in serum levels of ALT and total calcium.

Elevated significant serum urea levels were observed in the treated group compared to the significant decreased levels noticed in the positive group.

3.2.3 Effect on serum thyroid related hormones

Results in Table 3 showed the variable serum levels of T4 among groups in association to nearly constant values of serum TSH and serum T3 except in the group administrated CBZ/MMZ only in which T3 levels were increased significantly compared to the normal control group.

Serum T4 was decreased non-significantly in CBZ/MMZ administrated group compared to the control group. Also a significant decrease in serum T4 level was observed in the co-administrated group compared to CBZ/MMZ administrated group.

Non-significant decrease in serum T4 level was recorded in the group of rats administrated saline compared to normal control group.

3.3 Histopathological examinations

3.3.1 Histopathological effects on the liver

Light microscopic observation of the hepatic tissues of the control and saline groups showed normal hepatocytes with rounded prominent nuclei and eosinophic cytoplasm and few spaced hepatic sinusoids arranged in between the hepatic cords around the central veins (Figs. 2 and 3).

In the group receiving carbimazole drug, only the most pronounced histological abnormalities observed

Table 2 Effect of carbimazole drug and xylo-oligosaccharide co-administration on serum liver and kidney functions in female rats

Groups parameters	Carbimazole (positive)	Carbimazole and xylo-oligosaccarides co-administration (treated)	1% saline control	Normal Control
ALT (U/ ml)	11.62 ± 0.59^{c}	12.89 ± 0.69	12.65 ± 0.94	13.98 ± 1.41^{b}
AST (U/ml)	$45.00 \pm 5^{a,c,d}$	$54.5 \pm 1.5^{a,b}$	$64.5 \pm 0.2^{b,c,d}$	$55.33 \pm 5^{a,b}$
Total protein (g/dl)	$10.3 \pm 0.015^{a,c,d}$	$8.32 \pm 0.065^{a,b,c}$	$7.42 \pm 0.25^{b,c,d}$	$7.95 \pm 0.057^{a,b,d}$
Urea (mg/dl)	$16.75 \pm 5.06^{c,d}$	$69.06 \pm 12.42^{a,b,c}$	25.66 ± 11.64^{d}	$40.57 \pm 2.68^{b,d}$
Creatinine (mg/dl)	$1.185 \pm 0.002^{a,c,d}$	0.77 ± 0.148^{b}	0.925 ± 0.003^{b}	0.931 ± 0.031^{b}
Total calcium (mg/dl)	15.15 ± 0.25^{c}	14.50 ± 0.30	13.55 ± 0.05	12.80 ± 2.00^{b}

P^a significant to 1% saline gp

P^b significant to positive gp

P^c significant to normal control gp

P^d significant to treated group

Table 3 Effect of carbimazole drug and xylo-oligosaccharides co-administration on thyroid related hormones in female rats

Parameters	Groups			
	Carbimazole (positive)	Carbimazole& and xylo-oligosaccarides co-administration (treated)	1% saline control	Normal Control
TSH (uIU/mL)	0.02 ± 0.01	0.015 ± 0.005	0.02 ± 0.001	0.02 ± 0.01
T4 (nmol/l)	81.30 ± 20.7^{t}	$39.95 \pm 4.15^{p,c}$	63.40 ± 11	99.30 ± 33.10^{t}
T3 (nmol/l)	$6 \pm 1.3^{c,t}$	2.45 ± 0.15^{p}	2.2 ± 0.10	2.59 ± 0.29^{p}

Data represented as mean ± S.D. *P* significant at $p \leq 0.05$

P^{p} significant to positive group while p^{c} significant to the normal control group

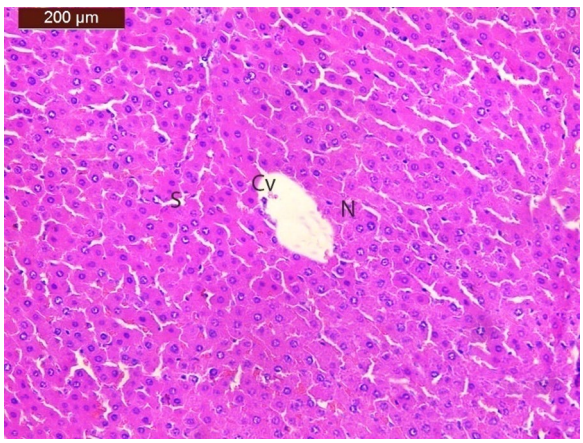

Fig. 2 A photomicrograph of rat liver of control group showing hepatic architecture. central vein (CV), hepatocytes (H), blood sinusoids (S) and nucleus (N)

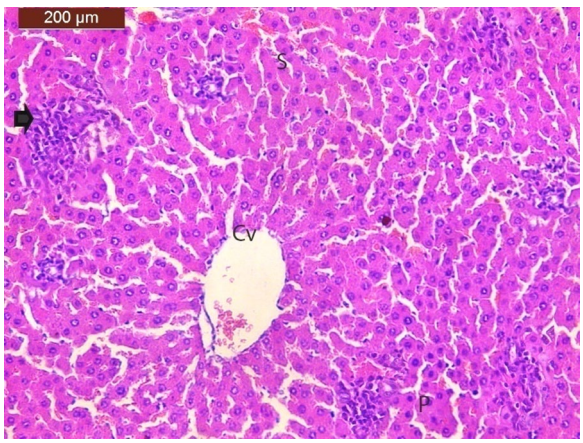

Fig. 4 A photomicrograph of rat liver of carbimazole drug only administrated group showing degeneration changes with eosinophilic cytoplasm necrotic are as associated with focal inflammatory cells (arrowhead) and dilated sinusoidal (S), deeply pyknotic nuclei (P)

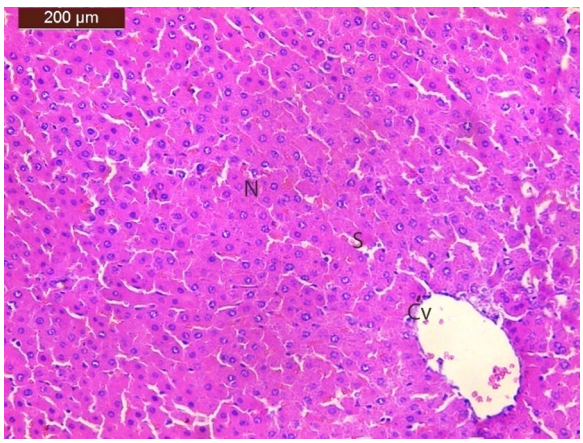

Fig. 3 A photomicrograph of rat liver of 1% saline control group showing hepatic architecture, central vein (CV), hepatocytes (H), blood sinusoids (S) and nucleus (N)

were degeneration changes with eosinophilic cytoplasm necrotic areas associated with focal mononuclear cell infiltration, and dilated sinusoidal, deeply pyknotic nuclei with mild activation of Kupffer cells (Fig. 4).

The liver of the group co-administrated carbimazole and xylo-oligosaccrides showed ameliorative effect with little degeneration of hepatocytes, enlargement of sinusoids and minimum activation of Kupffer cells (Fig. 5).

3.3.2 Histopathological effects on the kidney

The sections of the control and saline groups of kidneys showed normal structural of glomerulus, urinary space, renal tubules both proximal convoluted tubules and distal convoluted tubule (Figs. 6 and 7).

The histopathological examination section of the kidney tissues in rat administrated carbimazole drug only showed degenerative changes in of glomeruli and tubules. Shrinkage of large number of glomeruli, mild dilatation of urinary space and lymphocytic infiltration in the interstitium. Also, some renal tubules showed coagulation necrosis with pyknotic nuclei with diffused hemorrhage was observed (Fig. 8).

In section of the group, co-administrated carbimazole drug and xylo-oligosaccrides showed remarkable restoration and retained normal appearance of renal tubules and

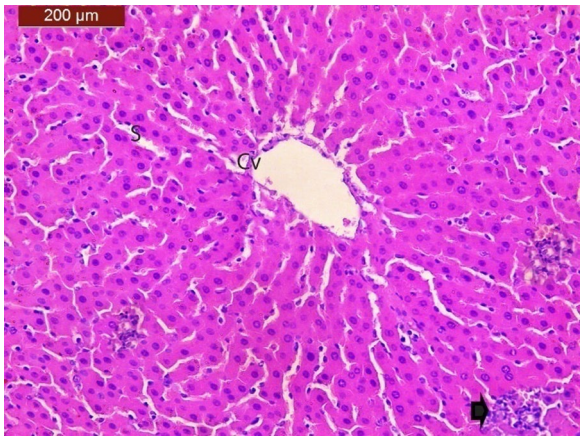

Fig. 5 A photomicrograph of rat liver of the co- administration of carbimazole drug and xylo-oligosaccride group showing ameliorative effect with few inflammatory cells (arrowhead), dialated sinusoids (S)

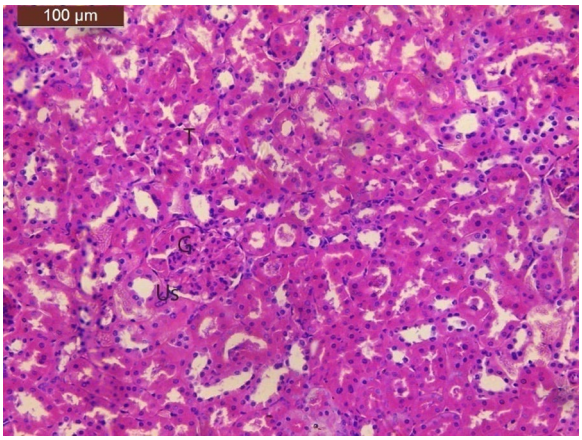

Fig. 7 A photomicrograph of rat kidney of 1% saline control group showing normal structure of the glomerulus (G), urinary space. (US) tubules (T)

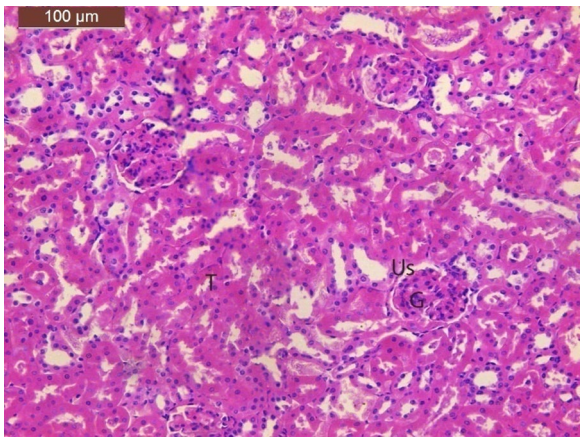

Fig. 6 A photomicrograph of rat kidney of control group showing normal structure of the glomerulus (G), urinary space. (US) tubules (T)

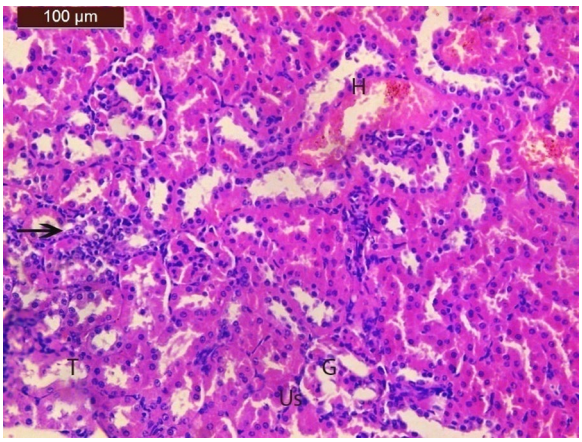

Fig. 8 A photomicrograph of rat kidney of carbimazole drug only administrated group showing degenerative changes in glomeruli and tubules, mild dilatation of urinary space and lymphocytic infiltration in the interstitium. Some renal tubules showed coagulation necrosis with diffused hemorrhage

glomerulus with a prominent urinary space with slight lymphocytic infiltration in the interstitium (Fig. 9).

3.3.3 Histopathological effects on the thyroid gland

The thyroid gland of the control and saline group, revealing normal thyroid architecture with variable follicles lined with cuboidal follicular epithelium with rounded nuclei and the follicular lamina, is filled with homogenous acidophilic colloid (Figs. 10 and 11).

The histopathological examination section of the thyroid gland tissues in rat administrated carbimazole drug only showed thyroid follicles with variable sizes. A decrease in colloidal material in some follicles and vacuolation in the cytoplasm of follicular cells and loss of their epithelium lining in addition to the presence of inflammatory cells aggregation and dark stained nuclei (pyknotic) are also noticed. In some follicles, multiple layers of follicular cells are seen (Fig. 12).

Section of thyroid gland of the group co-administrated with carbimazole and xylo-oligosaccharides showed nearly normal thyroid follicles with colloid in their lumina and some vacuolation in the cytoplasm of follicular cells and slight inflammatory cells. In some follicles, multiple layers of follicular cells are seen (Fig. 13).

3.3.3.1 Liver See Figs. 2, 3, 4 and 5.

3.3.3.2 Kidneys See Figs. 6, 7, 8 and 9.

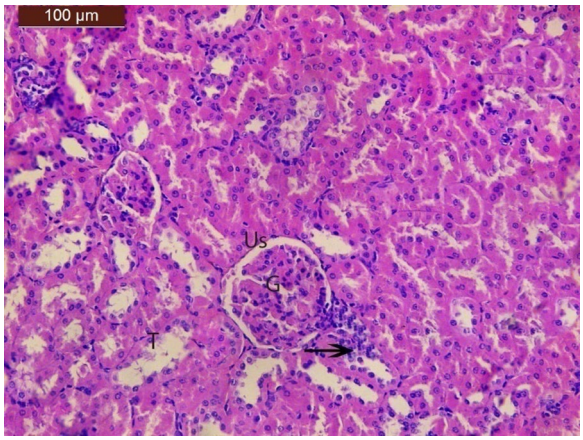

Fig. 9 A photomicrograph of rat kidney of the co-administration of carbimazole and xylo-oligosaccrides group showing remarkable restoration and the normal appearance of glomerulus and renal tubules with slight lymphocytic infiltration in the interstitium

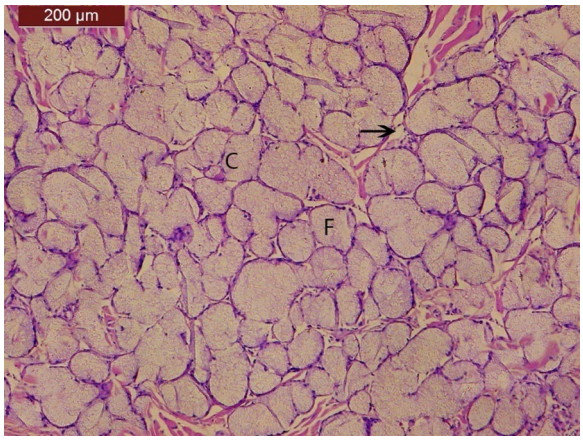

Fig. 11 Photomicrograph of section of thyroid gland of saline group revealing normal thyroid architecture with variable follicles lined with cuboidal follicular epithelium (F) with rounded nuclei. The follicular lamina is filled with homogenous acidophilic colloid (CO) blood vessels (arrow)

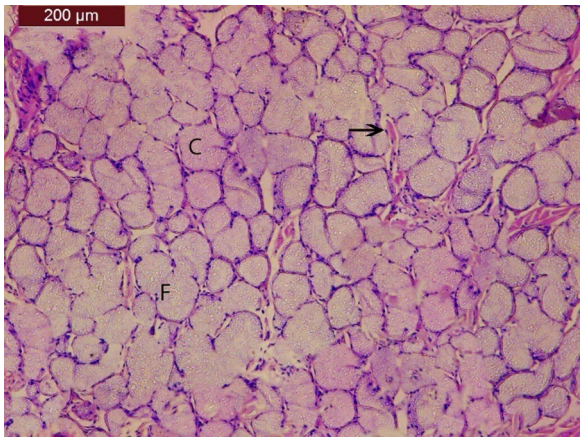

Fig. 10 Photomicrograph of section of thyroid gland of control group revealing normal thyroid architecture with variable follicles lined with cuboidal follicular epithelium (F) with rounded nuclei. The follicular lamina is filled with homogenous acidophilic colloid (CO) blood vessels (arrow)

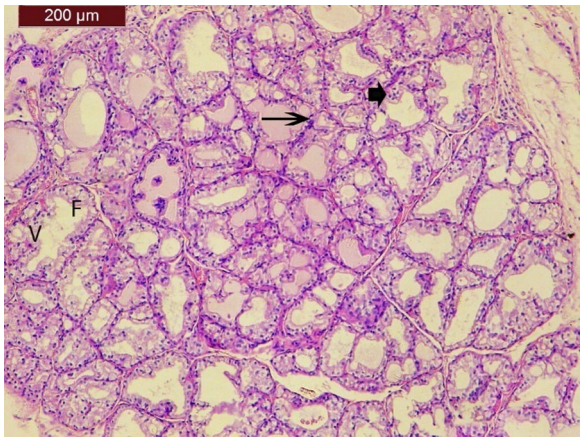

Fig. 12 Photomicrograph of section of thyroid gland of the group administrated carbimazole only showing thyroid follicles with variable size. A decrease in colloidal material in some follicles and vacuolation in the cytoplasm of follicular cells (V) and loss of their epithelium lining. Presence of inflammatory cells aggregation and dark stained nuclei (pyknotic) (arrow). In some follicles, multiple layers of follicular cells are seen (arrowhead)

3.3.3.3 Thyroid gland See Figs. 10, 11, 12, and 13.

4 Discussion

Negative effects resulting from administration of carbimazole (CBZ) are not limited to the patients only, but may extend to future generations also. As an example, hyperthyroid pregnant mothers who are undergoing treatment with CBZ expose their fetuses during pregnancy to the previously stated risks caused by the drug and these effects can extend after birth [27–31].

This highly sensitive pathological condition requires applying a safe and careful treatment for hyperthyroid mothers, while keeping their embryos without harm.

Another example that displayed the extended harmful effect of CBZ on the future generations was altering the structure and functions of the reproductive organs as observed in experimental animals [32, 33].

Our study introduced XOS, extracted from *Aspergillus terreus* xylanase degradation of xylan, which is considered a safe and cheap natural compound, for the first time

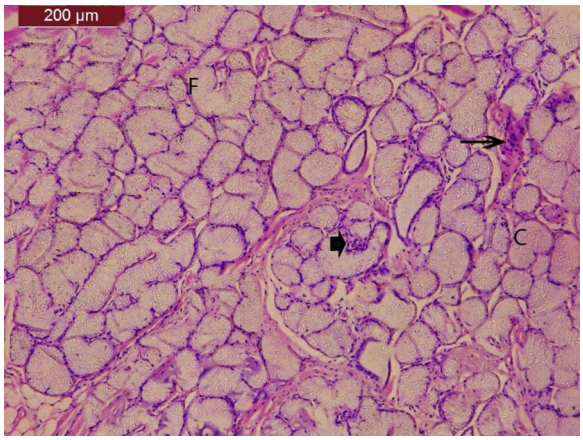

Fig. 13 Photomicrograph of section of thyroid tissues of the group co-administrated with carbimazole and xylo-oligosaccharides showing nearly normal thyroid follicles (F) with colloid in their lumina and slight inflammatory cells (arrow). In some follicles, multiple layers of follicular cells are seen (arrowhead)

to solve CBZ/MMZ life-threatening problems. Recently, XOS represent one of the beneficial oligosaccharides due to its great functional properties. XOS are characterized by exerting a prebiotic activities, low calories and high stability [18]. They were used as a food ingredient for improving gastrointestinal health in Japan since 1990 and its safe use as an additive to food or as a supplement is applied in many countries [34–36].

Observing the reaction of the body organs in the early stages during the use of the drug therapy can be an important step to control and prevent its side effects. Three-week period of time was preferred in the present work to observe the altered parameters in the body in the early stages during CBZ/MMZ administration and trace the effect of the co-administration of xylo-oligosaccharides in treating them.

In the present work, CBZ /MMZ administrated group showed a significant increase in body weight gain (28%) in comparison with the normal control group which displayed a significant increase (26%) compared to their corresponding initial body weights (Table 1). There was also a significant increase in serum total protein content, creatinine and total calcium levels. On the other hand, a significant decrease in serum ALT, AST and urea levels was noticed in this group compared to the normal control group (Table 2). This pathological case which represented the liver and the kidneys dysfunction was assured by observing the histological examinations (Figs. 4 and 8).

Ahmed et al. [16] reported non-significant change in body weights of MMZ-induced hypothyroid rats at the end of a month from administration and reported the changes in the lipid profile in rats.

The liver is one of the main organs that is affected by CBZ administration in humans [37, 38] and animals. Heidari et al. [39] reported that MMZ administrated mice showed significant elevation in serum ALT levels after five hours from administration with respect to control mice group which reflected the liver injury. They also discussed the role of antioxidants in preventing MMZ-induced liver injury. Akai et al. [40] discussed the role of liver Kupffer cells-mediated immune responses in increasing the liver injury complications due to MMZ administration.

Our results represented different data from the previous authors and also from Kadhim et al. [13], Mosa et al. [14], Hussein et al. [41] and many others who reported a significant increase in serum levels of ALT, AST and urea in the group administrated the drug only for an average period of a month or two. Our results showed an expression of a lazy liver case in the group of rats administrated CBZ/MMZ only as there was a significant decrease in serum levels of ALT and AST besides the marked significant decrease in serum urea levels compared to the control group (Table 2).

These altered results can be attributed to the different circumstances of the experiment including the high dose used of the drug (83 mg/kg b.w) during the period of three weeks besides dissolving the drug in 1% saline rather than water. The tested parameters data in the rats group administrated 1% saline only showed slightly significant changes compared to the control group.

The significant changes reported in the tested parameters in CBZ dissolved in 1% saline group compared to the group administrated saline only showed the clear effect of the drug in the presence of 1% saline (Table 2).

Some studies discussed the relation between saline administration to experimental animals and the alteration in the liver and kidney functions. Li et al. [42] investigated that addition of 6% sodium chloride to mice diet for 6 weeks can alter the liver metabolism on the level of transcriptome level without altering the hepatocytes shape with no significant changes in serum ALT and AST. Bernardi et al. [43] observed the significant effects of 0.2, 1.2 and 8.2% sodium chloride content diets on body weights and rats' kidneys function after one month from performing of left uninephrectomy surgery. They reported that there was a decrease in rats' weight in case of rats administrated 8.2% NaCl diet and presence of kidney hypertrophy and an increase in tubular area in both administrated NaCl diets.

In our study, the liver and the kidneys were obviously affected by CBZ/MMZ administration (the positive group) and this appeared clearly in both of the biochemical results (serum parameters) and the histological findings (Table 2) (Figs. 4 and 8).

On the other hand, the great histological changes displayed in the thyroid tissue in case of the group administrated CBZ/MMZ only still were not detected clearly in the serum thyroid profile. The drug (positive) administrated group showed signs of degeneration in the thyrocytes; vacuolation in the cytoplasm of follicular cells and a decrease in colloidal material in addition to loss of the epithelium lining besides pyknotic nucleii and aggregation of inflammatory cells in the thyroid tissue. At the same time, signs of hyperplasia in some other follicular cells were also observed as there were multilayers of epithelial cells existed (Fig. 12). These results are parallel to those of Hossain [17] that confirmed thyroid hyperplasia and presence of thyrocytes distortions due to CBZ/MMZ administration to rabbits.

In our study, we can report the first step that clarified how the thyroid functions began to be changed slowly as a result of CBZ administration before leading to a hypothyroid case in rats. Rats still did not reach the hypothyroid case and there was a non-significant decrease in serum T4 compared to control group which may reflect the abnormal functions of the thyroid gland due to the observed structural changes. This result was built on the fact of producing T4 from the thyrocytes [44–46].

Serum normal TSH and a significant increased T3 level were detected. This increasing level of T3 was attributed to unbalanced and disrupted equilibrium of T3 level between the serum and body organs [47, 48] in CBZ/MMZ administrated group compared to the control one as shown in Table 3 and Fig. 12. In normal cases, most of the production of T3 exits by deiodinases in the target tissues [44–46].

So we can conclude that this disruption in T3 levels besides the decrease in serum T4 levels (due to thyroid structural distortion) will lead gradually to T3 serum decreasing levels which in turn causes a feedback mechanism that leads to an increase in serum TSH. Increased TSH levels failed to force the thyroid gland to produce hormones due to its structural disruption causing an extended hypothyroid case.

Our results can be considered as an interpretation to those reported by Cakic-Milosevic et al. [49] and Ahmed et al. [16] as they observed a decrease in serum T4 and T3 in rats administrated 0.02% MMZ in drinking water for three and four weeks, respectively. Also Al-Shaikh et al.; Al-Naely and Shattnan [50, 51] and Lashein et al. [52] used CBZ/MMZ in inducing experimental hypothyroidism in rodents.

Hypothyrodism risk case is firstly preceded with a case of subclinical hypothyroidism that is characterized by significant increase in serum TSH with a normal levels of T4 and T3 [53, 54] which was not observed in our study.

The co-administration of XOS to CBZ/MMZ drug controlled significantly the increase in rats' body weights compared to the increase observed in the body weights of rat administrated CBZ/MMZ only with respect to their initial corresponding weights during the three weeks of the experiment. It also decreased significantly serum total protein, creatinine and AST levels besides the non-significant improvement in calcium and ALT levels compared to the CBZ/MMZ (positive) administrated group (Table 2).

The present work reported a significant increase in serum urea levels in the group co-adminstrated XOS and CBZ/MMZ compared to CBZ/MMZ positive group, and this case may be attributed to the presence of some impurities (nitrogenous wastes) found during the extraction of XOS (Table 2). The normal liver produces urea due to metabolizing proteins and nitrogenous containing compounds through performing the urea cycle. Urea is then eliminated through the kidneys [55].

The histological examinations reflected the improvement in the liver and kidneys functions (Figs. 5 and 9) compared to CBZ/MMZ only administrated group (Figs. 4 and 8).

With respect to the thyroid gland, XOS co-administration to CBZ/MMZ drug showed a normal restored level of serum T3 and significant decrease in serum T4 compared to the drug group.

This decrease in T4 level can be attributed to the great affinity (low dissociation rates) of thyroxine binding proteins (TBP) toward thyroxine (T4) that can exists as a reaction against an abnormal or a disease case and formation of a tightly bounded thyroxine reservoir [46, 56, 57]. Strong binding of T4 to plasma proteins may display wrong results when determined in laboratory which then leads to false therapy [58, 59].

Adult healthy rodents have two types of thyroid hormones (THs) binders: transthyretin (TTR) and albumin. They are also known as thyroid hormones distributers as they control and distribute (THs) in blood and cerebrospinal fluid to diffuse freely. These protein distributors are also found in human serum with different affinities toward (THs) in addition to thyroxin binding globulin (TBG) [58, 59] which exits in rats only in juveniles and old rats and not in adults [60].

Bounded T4 and T3 in serum act as a buffering media and control the concentration of the freeT4 and free T3 that in turn enter the different types of the body cells and exerts its genomic or non genomic actions [61, 62].

The histological findings of the group co-administrated XOS to CBZ clarified the great influence of XOS extracted from *Aspergillus terreus* xylanase degradation of xylan on restoring the thyrocytes structure and function on the short run besides those detected in

the liver and kidneys. This result can be considered as a new important application added to XOS numerous benefits.

The results may discuss the beneficial and safe use of xylo-oligosacchrides for enhancement of the general body health during CBZ/MMZ administration.

Our results are in accordance with those of Spiljar et al.; Li et al. and Yan et al. [18, 63, 64]. Their researches discussed the beneficial mechanisms and effects of XOS extracted from different sources in controlling body weights through reducing visceral fat, regulating the disorders of liver lipid metabolism and protecting from oxidative stress associated to fat diets.

5 Conclusion

Xylo-oligosaccharides extracted from *Aspergillus terreus* xylanase degradation of xylan ameliorated the complications caused due to CBZ/MMZ administration. XOS kept the body weight of rats in the normal range and improved the liver and the kidney functions in addition to its important role in restoring the thyroid gland normal shape and functions.

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
DNSA	3,5-Dinitrosalicylic acid
KH_2PO_4	Potassium dihydrogen phosphate
T3	Tri-iodothyronine
T4	Tetra-iodothyronine (thyroxine)
TSH	Thyroid-stimulating hormone
CBZ	Carbimazole drug
MMZ	Methimazole
XOS	Xylo-oligosaccharides

Acknowledgements

Not applicable.

Author contributions

SAN extracted xylo-oligosaccharides, and DSF put the experimental design, performed the practical work and wrote the manuscript.

Funding

This work was not supported by any external or internal funds.

Availability of data and materials

All the obtained data in the present work are reported in this published article.

Declarations

Ethics approval and consent to participate

The experiment was performed under the approval of the medical ethical committee of the National Research Centre (No. 18157).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 27 January 2023 Accepted: 11 August 2023
Published online: 29 August 2023

References

1. Foda DS, Ibrahim NE (2021) Detection of some hormonal and enzymatic disorders associated to experimentally iodine induced hyperthyroidism. Egypt J Chem 64(4):1789–1798
2. Sakr SA, El Naby SEH, Okdah YA, El-Shabka AM (2017) Impact of ginger aqueous extract on carbimazole induced testicular degenerative alterations and oxidative stress in albino rats. J Coast Life Med 5(4):167–173
3. Bashandy MA (2018) Role of rosemary (*Rosmarinus officinalis*) extract on carbimazole induced alteration in pancreas of adult male albino rat (histological, immuno-histochemicaland ultrastructural study). J Am Sci 14(1):110–124
4. Khatab MM, Hulail MAS, HegazyAA MHO (2018) Study of toxic effect of methimazole on the cortical structure of adult male albino rats kidneys and the ameliorated effect of thyroxin. Zagazig Univ Med J 24(3):208–219
5. Abdelrahman MM (2019) Chromatographic methods development, validation and degradation characterization of the antithyroid drug Carbimazole. Biomed Chromatogr 33:e4472. https://doi.org/10.1002/bmc.4472
6. Ronchetti R, Moroni G, Carotti A, Gioiello A, Camaioni E (2021) Recent advances in urea- and thiourea-containing compounds: focus on innovative approaches in medicinal chemistry and organic synthesis. RSC Med Chem 12(7):1046–1064. https://doi.org/10.1039/d1md00058f
7. Knudsen N, Laurberg P, Rasmussen LB, Bulow I, Perrild H, Ovesen L, Jørgensen T (2005) Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 90(7):4019–4024
8. Dale J, Daykin J, Holder R, Sheppard MC, Franklyn JA (2001) Weight gain following treatment of hyperthyroidism. Clin Endocrinol 55(2):233–239
9. Jonklaas J, Nsouli-Maktabi H (2011) Weight changes in euthyroid patients undergoing thyroidectomy. Thyroid 21(12):1343–1351
10. Kyriacou A, Kyriacou A, Makris KC, Syed AA, Perros P (2019) Weight gain following treatment of hyperthyroidism—a forgotten tale. Clin Obes 9(5):e12328. https://doi.org/10.1111/cob.12328
11. Kobayashi M, Higuchi S, Ide M, Nishikawa S, Fukami T, Nakajima M, Yokoi T (2012) Th2 cytokine-mediated methimazole-induced acute liver injury in mice. J Appl Toxicol 32(10):823–833
12. Karalus M, Jade Tamatea AU, Conaglen JV, Elston MS (2016) Carbimazole-induced hepatotoxicity—avoid rechallenging. JSM Thyroid Disord Manag 1(1):1001
13. Kadhim SH, Musa AU, Al-Kareem ZA, Ubaid MM, Aziz ND (2018) The analysis of the protective feature of *Nigella sativa* in reducing carbimazole toxicity including liver and kidney parameters on Albino male rats. Sci J Med Res 2(5):14–18
14. Mosa AU, Naser IH, Khalaf BH (2021) The protective effect of boswellia against carbimazole incuced reanl and hepatic toxicity on male rats. Int J Pharm Res 13(1):1316–1324
15. Wu TY, Wang CH, Tien N, Lin CL, Chu FY, Chang HY, Lim YP (2020) A population-based cohort study on the association of hyperthyroidism with the risk of hyperlipidemia and the effects of anti-thyroid drugs on hepatic gene expression. Front Med (Lausanne) 7:228. https://doi.org/10.3389/fmed.2020.00228
16. Ahmed OM, Abdel Gabbar M, Ali TM (2012) Impacts of the coexistence of diabetes and hypothyroidism on body weight gain, leptin and various metabolic aspects in albino rats. J Diabetes Complicat 26(6):491–500. https://doi.org/10.1016/j.jdiacomp.2012.05.021
17. Hossain AO (2019) Carbimazole and its effects on thyroid gland of female rabbits. J Pharm Sci Res 11(4):1610–1613
18. Yan F, Tian S, Chen H, Gao S, Dong X, Du K (2022) Advances in xylooligosaccharides from grain byproducts: extraction and prebiotic effects. Grain Oil Sci Technol 5(2):98–106. https://doi.org/10.1016/j.gaost.2022.02.002
19. Nour SA, El-Sayed GM, Taie HAA, Emam MTH, El-Sayed AF, Salim RG (2022) Safe production of *Aspergillus terreus* xylanase from *Ricinus communus*: gene identification, molecular docking, characterization, production of xylooligosaccharides and its biological activities. J Genetic Eng Biotechnol 20(121):1–26

20. Bailey MJ, Biely P, Poutanen K (1992) Interlaboratory testing of methods for assay of xylanase activity. J Biotechnol 23(3):257–270
21. Lowry OH, Rosebrough NJ, Fan AC, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275
22. Adachi S (1965) Thin-layer chromatography of carbohydrates in the presence of bisulfite. J Chromatogr A 17:295–299
23. Moure A, Gullon P, Dominiguez H, Parajo JC (2006) Advances in the manufacture, purification and applications of xylooligosaccharides as food additives and nutraceuticals. Process Biochem 41(9):1913–1923
24. Foda DS, Nour SA, Ismail SA, Hashem AM (2022) Safety evaluation of β-mannanase enzyme extracted from the mutant pathogenic fungal strain *Penicillium citrinum* in female Wistar rats. Int J Vet Sci 11(1):74–81. https://doi.org/10.47278/journal.ijvs/2021.074
25. Gindler M, King JD (1972) Rapid colorimetric determination of calcium in biologic fluids with methyl thymol blue. Am J Clin Pathol 58(4):376–382
26. Banchroft JD, Stevens AA, Turner DR (1996) Theory and Practice of histological techniques, 4th edn. Elsevier, Churchil Livingstone
27. Khan I, Okosieme OE, Lazarus JH (2017) Current challenges in the pharmacological management of thyroid dysfunction in pregnancy. Expert Rev Clin Pharmacol 10(1):97–109. https://doi.org/10.1080/17512433.2017.1253471
28. Francis T, Francis N, Lazarus JH, Okosieme OE (2020) Safety of antithyroid drugs in pregnancy: update and therapy implications. Expert Opin Drug Saf 19(5):565–576. https://doi.org/10.1080/14740338.2020.1748007
29. Morales DR, Fonkwen L, Nordeng HME (2021) Antithyroid drug use during pregnancy and the risk of birth defects in offspring: systematic review and meta-analysis of observational studies with methodological considerations. Br J Clin Pharmacol 87(10):3890–3900. https://doi.org/10.1111/bcp.14805
30. Agrawal M, Lewis S, Premawardhana L, Dayan CM, Taylor PN, Okosieme OE (2022) Antithyroid drug therapy in pregnancy and risk of congenital anomalies: systematic review and meta-analysis. Clin Endocrinol (Oxf) 96(6):857–868. https://doi.org/10.1111/cen.14646
31. Liu Y, Li Q, Xu Y, Chen Y, Men Y (2023) Comparison of the safety between propylthiouracil and methimazole with hyperthyroidism in pregnancy: a systematic review and meta-analysis. PLoS ONE 18(5):e0286097. https://doi.org/10.1371/journal.pone.0286097
32. Prathima P, Venkaiah K, Pavani R, Daveedu T, Munikumar M, Gobinath M, Valli M, Sainath SB (2017) α-Lipoic acid inhibits oxidative stress in testis and attenuates testicular toxicity in rats exposed to carbimazole during embryonic period. Toxicol Rep 4:373–381. https://doi.org/10.1016/j.toxrep.2017.06.009
33. -Prathima P, Venkaiah K, Pavani R, Rao KVLS, Pitchika GK, Kishori B, Dirisala VR, Pradeepkiran JA, Sainath SB (2023) Transcriptomic profiling identified altered expression of genes associated with testicular functions in adult F1 rats exposed to carbimazole during fetal period. J Proteomics, 274.
34. Mäkeläinen H, Juntunen M, Hasselwander O (2009) Prebiotic potential of xylo-oligosaccharides. In: Charalampopoulos D, Rastall RA (eds) Prebiotics and probiotics science and technology. Springer, New York. https://doi.org/10.1007/978-0-387-79058-9_8
35. Hijová E, Bertková I, Štofilová J (2019) Dietary fibre as prebiotics in nutrition. Cent Eur J Public Health 27(3):251–255. https://doi.org/10.21101/cejph.a5313
36. Huang C, Yu Y, Li Z, Yan B, Pei W, Wu H (2022) The preparation technology and application of xylo-oligosaccharide as prebiotics in different fields: A review. Front Nutr 9:996811. https://doi.org/10.3389/fnut.2022.996811
37. Akmal A, Kung J (2014) Propylthiouracil, and methimazole, and carbimazole-related hepatotoxicity. Expert Opin Drug Saf 10:1397–1406. https://doi.org/10.1517/14740338.2014.953796
38. Wang MT, Lee WJ, Huang TY, Chu CL, Hsieh CH (2014) Antithyroid drug-related hepatotoxicity in hyperthyroidism patients: a population-based cohort study. Br J Clin Pharmacol 78(3):619–629. https://doi.org/10.1111/bcp.12336
39. Heidari R, Babaei H, Roshangar L, Eghbal MA (2014) Effects of enzyme induction and/or glutathione depletion on methimazole-induced hepatotoxicity in mice and the protective role of N-acetylcysteine. Adv Pharm Bull 4(1):21–28
40. Akai S, Uematsu Y, Tsuneyama K, Oda S, Yokoi T (2016) Kupffer cell-mediated exacerbation of methimazole-induced acute liver injury in rats. J Appl Toxicol 36(5):702–715. https://doi.org/10.1002/jat.3202
41. Hussein Naser I, Aziz ND, Abed Al-Kareem Z (2022) The protective effect of turmeric against carbimazole induced toxicity in male rats. Arch Razi Inst 77(6):2081–2087. https://doi.org/10.22092/ARI.2022.358130.2156
42. Li Y, Lyu Y, Huang J et al (2021) Transcriptome sequencing reveals high-salt diet-induced abnormal liver metabolic pathways in mice. BMC Gastroenterol 21:1–10. https://doi.org/10.1186/s12876-021-01912-4
43. Bernardi S, Toffoli B, Zennaro C, Tikellis C, Monticone S, Losurdo P, Bellini G, Thomas MC, Fallo F, Veglio F, Johnston CI, Fabris B (2012) High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage. Nephrol Dial Transplant 27(5):1793–1800. https://doi.org/10.1093/ndt/gfr600
44. Harvey CB, Williams GR (2002) Mechanism of thyroid hormone action. Thyroid 12:441–446
45. Damiano F, Rochira A, Gnoni A, Siculella L (2017) Action of thyroid hormones, T3 and T2, on hepatic fatty acids: differences in metabolic effects and molecular mechanisms. Int J Mol Sci 18(744):1–19. https://doi.org/10.3390/ijms18040744
46. Ceccarini G, Santini F, Vitti P (2017) Tests of thyroid function. In: Vitti P, Hegedus L (eds) Thyroid diseases. Endocrinology. Springer, Cham. https://doi.org/10.1007/978-3-319-29195-6_2-1
47. Boelen A, van der Spek AH, Bloise F, de Vries EM, Surovtseva OV, van Beeren M, Ackermans MT, Kwakkel J, Fliers E (2017) Tissue thyroid hormone metabolism is differentially regulated during illness in mice. J Endocrinol 233(1):25–36. https://doi.org/10.1530/JOE-16-0483
48. Salas-Lucia F, Bianco AC (2022) T3 levels and thyroid hormone signaling. Front Endocrinol (Lausanne) 13:1044691. https://doi.org/10.3389/fendo.2022.1044691
49. Cakic-Milosevic M, Korac A, Davidovic V (2004) Methimazole-induced hypothyroidism in rats: effects on body weight and histological characteristics of thyroid gland. Jugosl Med Biohemija 23(2):143–147
50. Al-Shaikh MN, Wahab TALA, Kareem SHA (2014) Hypothyroidism induced by carbimazole in diabetic mice and its management using Parsley and *Eruca sativa* oil. IOSR J Pharm Biol Sci 9(1):24–27
51. Al-Naely AJ, Shattnan DT (2017) Effect of grape seed extract on biochemical factor and histological changes in liver and the kidney in albino rat infected hypo-hyperthyroidism induced laboratory by carbimazole and L-thyroxine. J Glob Pharma Technol 9(9):174–181
52. Lashein FED, Abd El Rehim S, Abu Amra EHSS (2022) Ameliorative effects of BPF separated from honey bee venoms on liver and kidney functions in hypothyroidic male rat's model. Sohag J Sci 7(3):61–70. https://doi.org/10.21608/sjsci.2022.143417.1004
53. Azim S, Nasr C (2019) Subclinical hypothyroidism: when to treat. Cleve Clin J Med 86(2):101–110
54. Leng O, Razvi S (2019) Hypothyroidism in the older population. Thyroid Res 12:2
55. Qiu J, Thapaliya S, Runkana A, Yang Y, Tsien C, Mohan ML, Narayanan A, Eghtesad B, Mozdziak PE, McDonald C, Stark GR, Welle S, Naga Prasad SV, Dasarathy S (2013) Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-κB-mediated mechanism. Proc Natl Acad Sci 110(45):18162–18167. https://doi.org/10.1073/pnas.1317049110
56. Janssen ST, Janssen OE (2017) Directional thyroid hormone distribution via the blood stream to target sites. Mol Cell Endocrinol 458:16–21. https://doi.org/10.1016/j.mce.2017.02.037
57. Bartalena L, Piantanida E (2019) Serum thyroid hormone-binding proteins. In: Huhtaniemi I, Martini L (eds) Encyclopedia of endocrine diseases, 2nd edn. Academic Press, Oxford, pp 442–447
58. Pappa T, Ferrara AM, Refetoff S (2015) Inherited defects of thyroxine-binding proteins. Best Pract Res Clin Endocrinol Metab 29(5):735–747. https://doi.org/10.1016/j.beem.2015.09.002
59. Mimoto MS, Refetoff S (2020) Clinical recognition and evaluation of patients with inherited serum thyroid hormone-binding protein mutations. J Endocrinol Invest 43(1):31–41. https://doi.org/10.1007/s40618-019-01084-9
60. Alshehri B, D'Souza DG, Lee JY, Petratos S, Richardson SJ (2015) The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. J Neuroendocrinol 27:303–323. https://doi.org/10.1111/jne.12271
61. Schreiber G (2002) The evolutionary and integrative roles of transthyretin in thyroid hormone homeostasis. J Endocrinol 175(1):61–73. https://doi.org/10.1677/joe.0.1750061

62. Rabah SA, Gowan IL, Pagnin M, Osman N, Richardson SJ (2019) Thyroid hormone distributor proteins during development in vertebrates. Front Endocrinol 10:506. https://doi.org/10.3389/fendo.2019.00506
63. Spiljar M, Merkler D, Trajkovski M (2017) The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 8:1353. https://doi.org/10.3389/fimmu.2017.01353
64. Li F, Li Q, Zhang Y, Zhou X, Yi R, Zhao X (2021) Effects of xylooligosaccharides on lipid metabolism, inflammation, and gut microbiota in C57BL/6J mice fed a high-fat diet. Front Pharmacol. https://doi.org/10.3389/fphar.2021.791614

Publisher's Note