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Ameliorative Effects of Bradykinin Potentiating Factor Separated From Honey Bee Venom on Liver and Kidney of Hypothyroidic Male Rat's Model.

Soheir A. Abd El Rehim[x], Elsabry Abu Amr[x]a, Fakhr El Din M. Lashei[x]n, Shoaeb, H. S.

Zoology Department, Faculty of Science, Sohag University, Sohag, 82524, Egypt

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Abstract: Carbimazole drug is used to induce hypothyroidism in the animal model. Hypothyroidism indicates disturbances of the liver and kidney functions. Several trials of treatment the hypothyroidism either by synthetic drugs or natural products were conducted in recent years. Concomitantly, this work was conducted to evaluate the effects of levothyroxine as a drug used for the treatment of thyroid hormones deficiency (hypothyroidism) and bradykinin potentiating factor (BPF) separated from honey bee, *Apis mellifera*, venoms, on the liver and kidney functions in a model of hypothyroid male rats in comparative mannar. The results revealed that, hypothyroid group significantly raised plasma total and direct bilirubin, ALT, AST, total protein, albumin, urea, uric acid and creatinine levels. Also, histological observations of the liver and kidney tissues in hypothyroidic male rats showed different abnormalities in these organs. Moreover, PAS stained liver and kidney sections revealed a decrease in polysaccharides in carbimazole treated group compared to control group. Treated animals with levothyroxine or BPF and carbimazole co-treated groups with either levothyroxine or BPF improved the negative effects or complication of hypothyroidism on the male rat's liver and kidney at the level of biochemical and histological studies. Levothyroxine as a drug and BPF as natural product revealed nearly similar improvement in this study. In addition, BPF ameliorating the deleterious effects of hypothyroidism possibly by the direct effect of this factor, stimulate the synthesis of T3, T4, growth hormones and endogenous bradykinin.

Keywords: BPF, levothyroxine, carbimazole,liver function and kidney function.

1 Introduction

Thyroid hormones stimulates turnover of glucose and the cycling between glycogenolysis and gluconeogenesis [1]. These hormones have multiple effects on liver function including stimulation of enzymes regulating lipogenesis and lipolysis as well as oxidative processes [2]. Also, they stimulate protein turnover by stimulating both protein synthesis and degradation, in addition to increase gene expression with concomitant increases in protein synthesis and enzyme activity [3]. The liver is fundamental in metabolizing thyroid hormones, and hepatocytes are often affected in thyroid disease.

There are interactions between renal and thyroidal functions [4]. Disturbance of thyroid hormones lead to dysfunction in kidney, while renal disease can cause disturbance of thyroid. Renal disorders and thyroid dysfunction may co-exist with same causes [5]. Decrease of thyroid hormones can disturb blood pressure and this lead to decrease in glomerular filtration, hyponatremia, and change in the excretion of water.

Thionamides {propylthiouracil (PTU), benzylthiouracil, carbimazole and methimazole} are the most widely used anti-thyroid drugs. Adverse immunological effects, other than isolated granulocytopenia, have been described within anti-thyroid medication[6].Rash, fever, arthralgias and granulocytopenia are the most common clinical manifestations.In the liver, 3-carbethoxy methimazole (MMZ) derivative carbimazole (CBZ) rapidly metabolized to MMZ [7]. CBZ/MMZ hepatotoxicity is usually cholestatic as compared to hepatocellular dysfunction resulting from PTU [8]. Carbimazole is a widely used drug in treatment of thyrotoxic cases. Hypothyroidism is one of carbimazole complications during treatment [9].

Bee venom is considered as an effective healing agent that used for alleviating persistent pain and treating several ailments including different rheumatic disorders as inflammation and degeneration of connective tissue (different types of arthritis [10-12].It is an inflammation modulator that regulates pro-inflammatory cytokines in hepatocyte and liver fibrosis [13]. It inhibits the secretion of

* Corresponding author E-mail: flashein@yahoo.com

pro-inflammatory cytokines, and decreasing the elevated serum amino-transferase enzymes in different models of induced hepatic injury[14] .Melittin is a principle toxic peptide of bee venom (Apis mellifera). It has a strong anti-inflammatory aging and used as traditional medicine for treatment of different types of diseases including gastrointestinal tract [15]. In addition to that, they have a protecting effect against the liver and nephrotoxicity, those induced with either acrylamide or chips administration [16].

BPF7 separated from jellyfish, Cassiopia andromeda is used as a natural peptide in treatment of liver and kidney dysfunction induced by indomethacin in model ulcer animals [17].It is ameliorating the deleterious effects of indomethacin possibly by the direct effect of this factor which acts as a gastroprotective agent or indirect action through the stimulation of endogenous bradykinin which in turn enhances prostaglandins synthesis [18]. Also, it stimulates the release of prolactin, proliferation of the bone marrow, lymphopoietic cells and enhanced lymphoid organs [19].Bradykinin attenuates liver damage and fibrosis development in a rat model of chronic liver injury [20] , enhanced prostaglandin synthesis which is a key molecule that stimulates the complex array of ulcer healing mechanism [21,22]. BPFs enhances protein enzyme biosynthesis, DNA and carbohydrates in liver tissue [23] and protects the liver from the toxicity of patulin mycotoxin [24].

2 Results

Total bilirubin, direct bilirubin, ALT and AST:

The presented data in table (1) and figure (1) showed a highly significant increase (P<0.001) in total bilirubin, direct bilirubin, ALT and AST concentrationsafter treatment with carbimazole compared to control group. In levothyroxine and BPF groups, non-significant change were found in total bilirubin and direct bilirubin compared to control. A significant increase detected in ALT and AST compared to control in levothyroxine and BPF groups. Groups treated with carbimazole with levothyroxine or BPF showed a significant increase in total and direct bilirubin but non-significant change in ALT and AST compared to control group. All treated and co-treated groups showed a significant decrease in the studied parameters compared to carbimazole group (Tab. 1).

Table 1: Effect of BPF and Levothyroxine on plasma levels of total and direct bilirubin, and the activities of ALT and AST of male albino rats induced by carbimazole for 60 days in different groups.

parameters		Control	Car	Levo	BPF	Car + Levo	Car + BPF
Total bilirubin mg / dL	Mean ± SE	0.65±0.3	4.68^{a**}±0.26	0.52^{b**}±0.05	0.75^{b**}±0.34	2.0^{ab**}±0.15	1.18^{ab**}±0.18
	% of change (1)		+620	-20	+15.3	+207	+81.5
	% of change (2)			-88	-83.9	-57.3	-74.7
Direct bilirubin mg / dL	Mean ±SE	0.25±0.05	1.5^{a**}±0.18	0.23^{b**}±0.04	0.29^{b**}±0.05	0.6^{ab**}±0.06	0.56^{ab**}±0.08
	% of change (1)		+500	-8	+16	+140	+124
	% of change (2)			-78	-80.6	-60	-62.6
ALT mg/dL	Mean ±SE	28.5 ±2.0	91.3^{a**}±2.6	39.4^{a*b**}±3.4	38.2^{a*b**}±1.9	33.3^{b**}±2.7	31.2^{b**}±2.4
	% of change (1)		+220.5	+37.9	+34	+16.8	+9.5
	% of change (2)			-56	-58.2	-63.5	-65.8
AST mg/dL	Mean ±SE	38.75±2.1	101.6^{a**}±2.4	55.6^{ab*}±4.7	57^{ab*}±3.1	41^{b**}±3.4	45^{b**}±1.06
	% of change (1)		+162.2	+43.5	+47	+5.8	+16
	% of change (2)			-45.3	-43.8	-59.6	-55.7

Significant difference between control and different groups. N=8.
* = P<0.05 Significant. ** = P<0.001 Highly Significant. Non-SignificantP>0.05
% of change (1) different from normal control group G1.
% of change (2) different from carbimazole group G2.
a=significant different from control group.
b= significant different from carbimazole group.
Carbimazole(Car),Levothyroxin(Levo),Bradykinin Potentiating Factor (BPF)

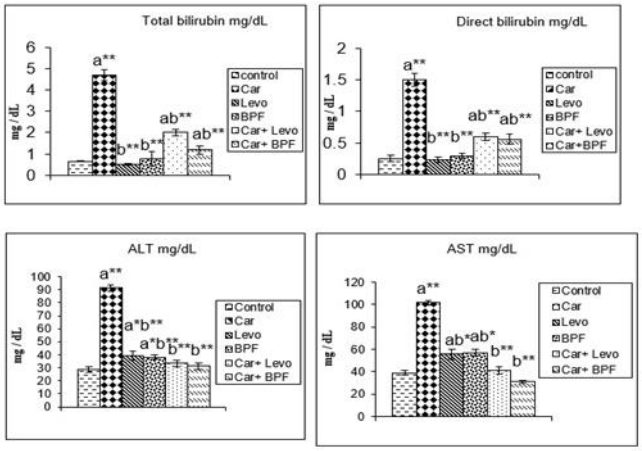


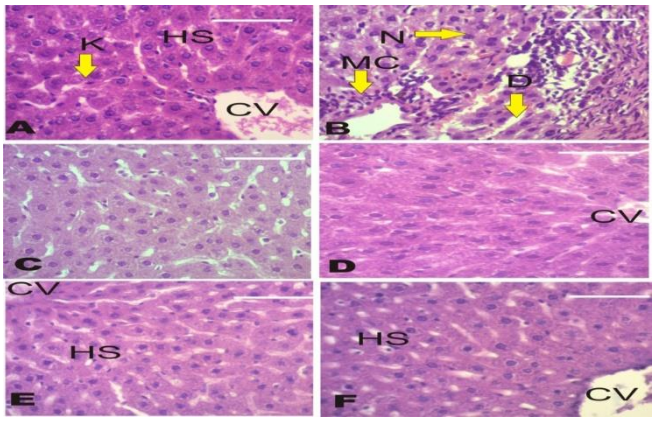
Figure 1: Effect of BPF and levothyroxine on plasma levels of total and direct bilirubin, and the activities of ALT and AST of male albino rats induced by carbimazole for 60 days in different groups. Carbimazole (Car), Levothyroxin (Levo), Bradykinin Potentiating Factor (BPF)

Histological study:

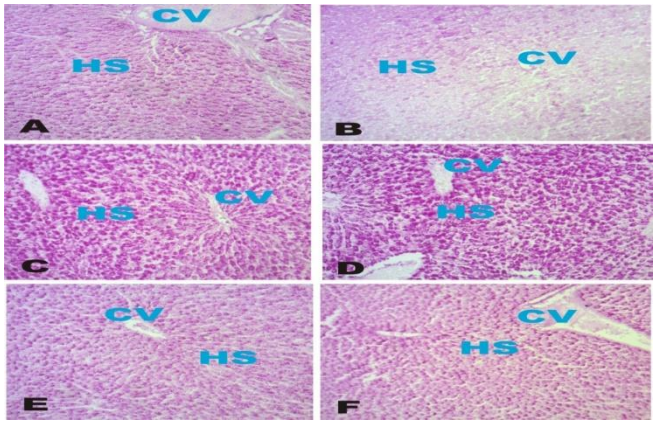
Histological observation of the liver section from the rats showing normal histological structure of hepatic lobules with central vein, Kuffer cells were recognized among the sinusoides in the control, the BPF and levothyroxine-treated animals (Pl. 1 A, C, D). In carbimazole-administrated animals, focal areas of necrosis with infiltrated cells were recognized (Pl. 1 B). The liver section from rats of the groups (G 2-2, G2-3) which were co-treated with BPF showed more normal appearance of the central vein and surrounding hepatocytes compared to both the control, BPF- and levothyroxine co-treatment, respectively (Pl. 1 E, F).

PAS- stained liver sections revealed a negative content

of polysaccharides in carbimazole- treated group (Pl. 2 B) as compared to control (Pl. 2 A). In BPF- and levothyroxine-treated animals (Pl. 2 C, D) and also, in carbimazole co-administration either with BPF or levothyroxine(Pl. 2 E, F), an increase of carbohydrate content of liver tissue was noted as compared to both carbimazole treated animals and control group.



Pl. 1:- Photomicrograph of liver tissue showing focal areas of cells infiltration with mononuclear cells (MC) in carbimazole group of daily administration (B) compared to control (A), BPF (C) and levothyroxine (D). Recovered tissue was noted in (E) or (F) of co- administration than in corresponding of carbimazole **H&E stain,scale bar 20µm. (**Hs, hepatic cords; CV, central vein).



PL. 2:-Photomicrograph of liver tissue showing negative contents of carbohydrates in carbimazole- treated group (B) compared to control (A). Intense staining of PAS in (C) and (D). Recovered carbohydrate content was noted in co- administration with both BPF (E) or levothyroxine (F) in carbimazole-treated groups. **. (**Hs, hepatic cords; CV, central vein). **PAS stain, scale bar 20µm**

Total protein, albumin, urea, uric acid and creatinine:

In table (2) and figure (2), a highly significant increase (P<0.001) in carbimazole treated group in all the tested parameters compared to control group. In levothyroxine, BPF and two co-administrated groups either with levothyroxine or BPF, a non-significant change (P>0.05) detected in all the previous parameters compared to control group. All treated and co-treated groups showed a significant decrease and improvement in previous parameters compared to carbimazole group.

Parameters		Control	Car	Levo	BPF	Car+Levo	Car+BPF
Total Protein mg / dL	Mean ±SE	6.8±0.72	10.8^{a**}±0.77	5.9^{b**}±0.74	5.4^{b**}±0.65	7.03^{b*}±0.63	6.05^{b*}±0.76
	% of change (1)		+58.8	-13.2	-20.5	+3.4	-11
	% of change (2)			-45.4	-50	-34.9	-43.9
Albumin mg / dL	Mean ±SE	4.8±0.74	8.7^{a**}±0.68	3.9^{b**}±0.44	4.3^{b**}±0.7	5.75^{b*}±0.78	4.2^{b**}±0.54
	% of change (1)		+81.25	-17.7	-10.4	-19.79	-12.5
	% of change (2)			-54.6	-51	-33.9	-51.7
Urea mg / dL	Mean± SE	27.26±2.25	54.3^{a**}±3.5	30.6^{b*}±2.3	32^{b*}±3.17	26.2^{b**}±1.9	31.5^{b*}±3.04
	% of change (1)		+99.2	+12.4	+17.4	-4	+15.5
	% of change (2)			-43.6	-40	-52	-41.9
Uric acid mg / dL	Mean ±SE	4.7±0.48	8.25^{a**}±0.64	3.9^{b**}±0.57	5.4^{b*}±0.38	5.3^{b*}±0.43	4.2^{b*}±0.72
	% of change (1)		+75.5	-17	+14.8	+12.7	-11.1
	% of change (2)			-52.7	-34.5	-35.7	-49.3
Creatinine mg/dL	Mean ±SE	0.62±0.03	1.94^{a**}±0.12	0.59^{b**}±0.02	0.73^{b**}±0.04	0.49^{b**}±0.1	0.5^{b**}±0.1
	% of change (1)		+223	-4.8	+17.7	-20.9	-19.3
	% of change (2)			-69.5	-62.4	-74.7	-74.0

Table 2 : Effect of BPF and levothyroxine on plasma total protein, albumin, urea, uric acid and creatinine concentrations of male albino rats induced by carbimazole for 60 days in different groups.

Significant difference between control and different groups. N=8.
* = P<0.05 Significant. ** = P<0.001 Highly Significant. Non-SignificantP>0.05
% of change (1) different from normal control group G1.
% of change (2) different from carbimazole group G2.
a=significant different from control group.
b= significant different from carbimazole group.
Carbimazole(Car),Levothyroxin(Levo),
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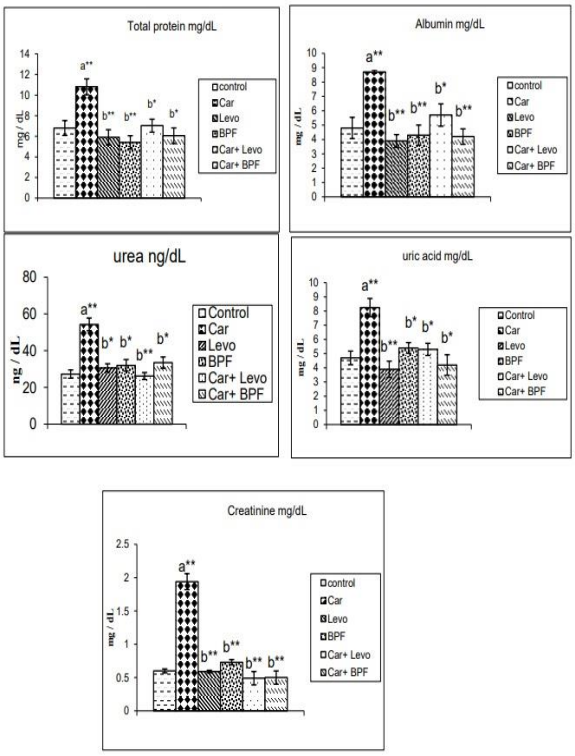


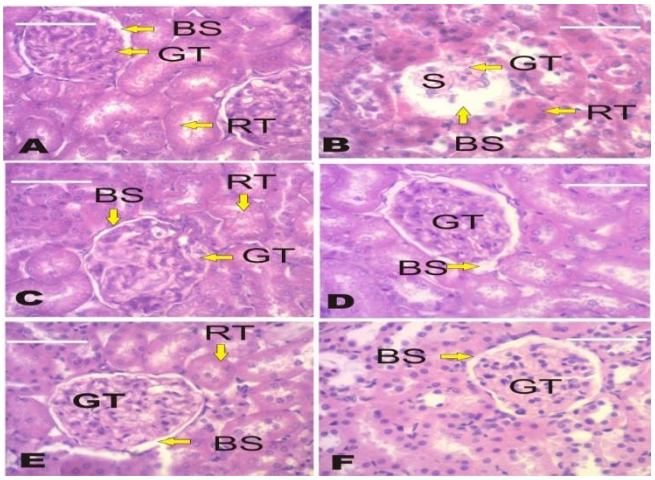
Figure 2: Effect of BPF and levothyroxine on serum total protein, albumin, urea, uric acid and creatinine levels of male albino rats induced by carbimazole for 60 days in different groups. Carbimazole(Car), Levothyroxin(Levo), Bradykinin Potentiating Factor (BPF)

Histological study

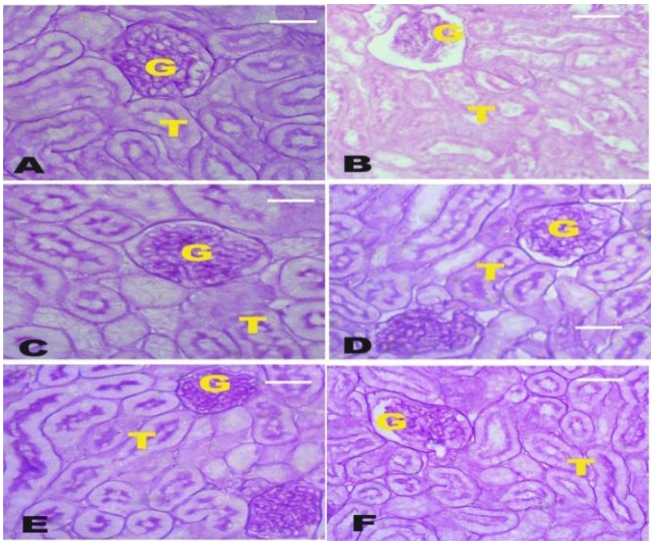
The kidney section of control group showing normal histological structure of glomeruli, glomerular tuft, Bowman's capsule and the renal tubules of the renal cortex in control, BPF and levothyroxine (Pl. 3 A, C, D).

The histological examination of the kidney section of the carbimazole- administrated group showed shrinkage at glomeruli tuft, and degeneration of epithelial cells of renal cortical tubules (Pl. 3 B). Co- administration of carbimazole group with either BPF or levothyroxine showing ameliorative effect of glomerular shrinkage (Pl. 3 E, F) as compared to carbimazole treatment.

In PAS- stained sections, the effect of carbimazole-administrated animals is clearly obvious as decreased in polysaccharides than control. Inhibition of mesangial and brush border stainability of the glomeruli and kidney tubules was noted (Pl. 4 B) as compared to the stainability of control (Pl. 4 A), BPF (Pl. 4 C) and levothyroxine (Pl. 4 D). In co- administration of carbimazole with BPF or levothyroxine groups, recovered stainability of mesangial cells and brush border of kidney tubules was noted (Pl. 4 E, F).



PL. 3: Photomicrographs of histological sections through the kidney cortex showing shrinked glomerular tuft (GT) of daily administrated carbimazole and degeneration of renal tubules (RT) compared to control (A), BPF (C) and levothyroxine (D) administrated groups. Amelioration of glomerular shrinkage of co- administration with either BPF (E) or levothyroxine (F) was noted. Bowman's capsule (BS), Glomerular tuft (GT), Renal tubule (RT). **H&E stain, scale bar 20µm**



PL. 4:- Photomicrographs of histological sections through the kidney cortex showing depressed stained mesangial and brush border of glomeruli (G) and kidney tubules (T) of carbimazole- administration compared to control (A), BPF (C) and levothyroxine (D) treated groups. Recovered stainability is shown in co- administration group with BPF (E) or levothyroxine (F) in carbimazole-administrated group. Glomerulus (G), tubule (T), **PAS stain, scale bar 20µm**

3 Discussion

Effect of carbimazole on liver and kidney function and structure:

Our results indicated that, experimental hypothyroid group induced by carbimazole revealed a significant increase in total and direct bilirubin levels compared to control group. This is could be due to decreased bilirubin UDP-glucuronyl-transferase activity resulting in a reduction in bilirubin excretion [25]. Also, hypothyroidism directly

affects the structure and function of hepatocytes, and is associated with disturbances excretion of bilirubin [**26**] . A significant increase detected in ALT and AST activities in hypothyroid group compared to control group are parallel with Christ-Crain et al.[**27**] who reported that the elevation of ALT and AST activities in overt hypothyroidism is associated with a decreased metabolic rate and a diminished catabolism of AST and ALT. Also, these findings were agreed with Ajayi and Akhigbe.[**28**] who explained that the damage which might happen to the hepatocytes in hypothyroid rats leads to leakage of enzymes from the cells.Similar results also were observed by Sakr et al. [**29**] who suggested that hypothyroidism caused hepatocyte damage due to free radicals and the inability of antioxidants to resist the increase in free radicals.

In this study, hypothyroid group showed a highly significant increase in total protein and albumin compared to control group. These results are parallel with Kondaveeti et al. [**30**] who suggested that, the increased albumin levels in hypothyroid patients are attributed to the deterioration of protein metabolism which further decreases turnover of proteins and increase of half-life of proteins.

Our results indicated that, model hypothyroid group induced by carbimazole showed significant increase in the levels of urea, uric acid and creatinine in blood plasma compared to control. These results are harmony with Shin et al. [**31**] who postulated that hypothyroidismis associated with reduced renal plasma flow and low glomerular filtration rate. Also, creatine kinase activity is increased in hypothyroidism, and this increase was related to decreased metabolism, excretion, or associated with hypothyroid-related myopathy[**32**]. Moreover, hyperuricemia leading to hypothyroid state and lead to significant increase in creatinine and uric acid levels [**33**].

In our study, carbimazole leads to degenerative changes such as congestion of blood vessels, appearance of inflammatory infiltrative cells and cytoplasmic vacuolization of the hepatocytes, some investigators using anti-thyroidal drugs such as carbimazole have reported similar side effects [**34**,**35**]. They reported that hypothyroidism induced by carbimazole leads to degenerative changes such as congestion of blood vessel, appearance of inflammatory infiltrative cells, cytoplasmic vacuolization of the hepatocytes and cell death. Also, the vacuolation of the hepatocytes cytoplasm may be occurring due to progressive ischemia, hypoxia and accumulation of lipid in the hepatocytes [**36**-**39**]

Saraji et al. [**40**] pointed out that areas of centrizonal necrosis were observed in liver histological sections of hypothyroid rats. The extracellular factors inducing cell death (such as tumor necrosis factor) increased in hypothyroid state causing damage to liver cells through the activation of an external pathway of apoptosis. Moreover, methimazole (anti-thyroidal drug) treatment has induced liver damage and showed abnormality in the enzymes of liver [**41**-**43**] in accordance with this statement the obtained results have confirmed that carbimazole is a hazard material that cause damage and abnormality to the liver function and structure [**44**].

As shown in the present results, histological examination of the kidney in hypothyroid group showed shrinkage at glomerular tuft and degeneration of epithelial cells of renal cortical tubules. These results are in consistent with Basuony. [**45**] who registered that the distal convoluted tubule is fewer in number and may be known by the pale cuboidal epithelial cells and William and Linda, [**46**] who reported that the distal convoluted tubules have a smooth internal surface, and lake brush border. Intake of drugs such as carbimazole has been shown to cause some tissues damage of the renal tubules [**47**].

Also, widened Bowman's space, congestion of glomeruli, abnormal shape of glomeruli, and area of hemorrhage in the interstitium between tubules were observed in carbimazole. Some kidney tubules had cytoplasmic vacuolations in the cells of lining and hyaline casts in lumen of many tubules. Inflammatory cells in the interstitium between tubules also appeared [**48**]. In addition to that, in hypothyroidism, excessive infiltration of inflammatory cells, expansion of renal tubules degeneration and clear necrosis of germicidal cells and clear congestion of blood vessels are seen [**49**].

For the histochemical effect of the hypothyroidism on the liver and kidney, this study revealed that, carbohydrates content was reduced in liver and kidney tissues. These results coordinate with those obtained by many investigators [**44**,**50**,**51**]. They reported that, the decrease in hepatic glycogen rate is due to the stimulation of key enzymes of glycogenolysis. Moreover, Salman et al.[**52**] reported that hypothyroidism decrease the stainability of carbohydrates in mesengial and brush border of glomeruli and kidney tubules.

Protective role of levothyroxine or BPF against hypothyroidism induced by carbimazole :

Levothyroxine or BPF treated groups showed a non-significant change in total and direct bilirubin, total protein, albumin and a significant increase in ALT and AST activities compared to control group. Carbimazole co-treated groups with either levothyroxine or BPF showed a significant improvement in all tested parameters. Moreover, this histological and histochemical observations in these groups showed normal histological structure and a significant increase of carbohydrate content of liver and kidney tissues as compared to both carbimazole and control groups. Improving biochemical parameters and liver structure may be attributed to the protective effect of levothyroxine therapy. In support of this assumption, levothyroxine drug used as a replacement for deficient

thyroid hormones [53] and it can reverses hormonal abnormalities in hypothyroidism [54,55]. Also, thyroid hormones stimulate growth hormone, control the metabolic rate and required for normal body growth [56-58], this leading to prevented liver damage from hypothyroid state and improved the liver function and structure.

With regard to the effect of BPF in previous biochemical parameters and hepatic tissues, the results are in agreement with Sakar et al. [59] and Hanafi et al. [60]. They reported that bee venom and its extracted factors reduce the inflammation and oxidative stress induced by carbimazole in albino rats. Also, the BPF enhanced the activities of the antioxidant enzymes [59] and induces insulin sensitivity which in turn ameliorate liver function and normalizing the redox state of the liver [60]. In addition, BPFs potentiate the endogenous BK, it inhances carbohydrate in liver tissue[23] , protects the liver from the toxicity of patulin mycotoxin [61] and ameliorating the deleterious effects of indomethacin in liver and kidney [17].

Therefore, the protective effects of BPF may be attributed to a direct effect of this natural product, activation of BK and stimulation of prostaglandin (PGs) which promoted the observed effects. In support of this, BK attenuate liver damage and fibrosis development in a rat model of chronic liver injury [20] and promoting liver regeneration [62,63]. Moreover, BK stimulates the release of PGs, Levant et al.[21] which improved liver function and prevented liver damage [64].

Treating animals with levothyroxine or BPF and carbimazole co-treated groups with either levothyroxine or BPF showed a non-significant change in the levels of the urea, uric acid and creatinine compared to control group, and a significant improvement in the previous tested parameters compared to carbimazole group. Also, the levothyroxine or BPF improved the complication of hypothyroidism on the kidney at the level on histological and histochemical studies. The protective effects of levothyroxine drug in all previous parameters are supported by Monzani et al. [65] and Ripoli et al. [66] who postulated that urea, uric acid and creatinine levels were normalized by levothyroxine. Also, Mooraki et al. [67] explained that aprogressive deterioration of renal function with severe hypothyroidism was reversed by L-thyroxine. Moreover, thyroid hormone treatment resulted in a rapid improvement of renal function and structure in hypothyroid patients with ischemic nephropathy [68].

Similarly, the BPF improved the deleterious effect of carbimazole on kidney function and structure, nearly as soon as levothyroxine in all tested previous parameters. These protective effects of BPF may be attributed to its antioxidant effects [69], inhibiting the secretion of pro-inflammatory cytokines, reduces the inflammation and oxidative stress, or may be result from the activation of bradykinin [23] and stimulation of prostaglandin synthesis [21], which correct or improved kidney function and structure. In support of this assumption, bee venom and its BPF protection the liver and kidney from toxicity were reported [70,71] .

Moreover, bee venom reduces the inflammation, and oxidative stress and it has a protective effect against impairment induced by gamma radiation in the liver and kidney tissues [72]. Also, bee venom and its BPF have a protective effect on renal tubular injury[73,74] and inhibiting the secretion of pro-inflammatory cytokines [14,16,74]

Conclusion

Levothyroxine as a drug and BPF as natural product revealed nearly similar improvement in this study. In addition, BPF ameliorating the deleterious effects of hypothyroidism possibly by the direct effect of this factor, stimulate the synthesis of T3, T4, growth hormones and endogenous bradykinin.

4 Experimental

Chemicals

Carbimazole was obtained from chemical industries Development (CID).Levothyroxine was purchased from Mercury pharma Group.

Bradykinin-potentiating factor (BPF)

Bee venom of Apis melifera was obtained from the Faculty of agriculture, Assuit University. Bradykinin potentiating factor (BPF) was isolated from bee venom according to the chemical methods of Ferreira [75]. In brief, bee venom was suspended in distelled water and absolute ethanol was added. The mixure was centrifuged and the supernatant fluid was evaporated under reduced pressure. The extract was washed three times with a mixure of 90% ethanol and diethyl ether (1 – 4; V:V) The precipitate was separated by centrifugation and dissolved in distelled water, then lyophilized. BPF was dissolved in 0.9 N of NaCl before using.

Animals

48 healthy adult male albino rats (180-200g) were brought from the farm of Zoology Department, Faculty of Science, Sohag University for experimentation. Animals were kept in the laboratory under normal conditions of light, temperature, humidity with access of food and water for two weeks then divided into 6 main and sub-groups.

Animal grouping

Animals were classified into four main groups. The first main group (G1) composed of 8 animals served as a negative control group. The second main group composed

of 24 animals administrated with carbimazole orally (0.05 mg/kg b.wt) daily according to Mustafa *et al*. [76] for 30 days inducing hypothyroidism. After that, this main group was divided into three sub-groups (G2-1, G2-2 and G2-3). G2-1 used as a positive control group (hypothyroid group). The sub-groups (G2-2 and G2-3) treated with levothyroxine (100µg/kg b.wt) and BPF (2.314 µg/kg b.wt) according to Abd Elazem *et al*. [77] and Abu-amra et al.[16], respectively. The third main group (G3) was administrated orally with levothyroxine daily (100µg/kg b.wt) for 30 days. The fourth main group (G4) composed of 8 animals injected intraperitonally (i.p.) with BPF (2.314 µg /kg b.wt) daily for 30 days.

Collection of samples

After another 30 days from treatment, the animals were sacrificed and dissected.The blood samples collected from the heart andtaken in a plastic tubes containing EDTA as anti-coagulant, then centrifuged at 3000 r.p.m for 20 minutes to obtain clear plasma for biochemical analysis.

From each animal, liver and kidney of studied groups were fixed in carnoy's fixative, dehydrated in ethyl alcohol, cleared in methyle benzoate, embedded in paraffin wax at 60c^{o} in oven for 6 hours, mounted in paraffin wax, cut at 7µ thick by microtome (Leica), the sections were mounted on the normal slides. Mounted sections were stained with hematoxylin and eosin for general histology[78] .Sections were dehydrated in ascending grade of ethanol, cleared in xylene and mounted with DPX. Sections of liver and kidney of studied groups were stained with periodic acid Schiffs (PAS) reaction for polysaccharides detection. The selected stained sections were photographed and processed as required.

Biochemical analysis

The biochemical parameters were assayed spectrophotometrically using commercially available specific kits purchased from Bio-diagnostic Company, Giza, Egypt. Total and direct bilirubin was determined according to Walter and Gerade [79]. The Plasma level of AST and ALT activities were determined spectrophotometrically using transaminases kits according to the method described by Young [80]. Total protein was detected according to Gornal et al. [81]. Albumin was determined according to Gendler [82]. Urea was measured according to Fawcett and Soctt [83]. Uric acid was measured according to Barham and Tinder [84]. Creatinine was measured according to Bartles and Larsen [85].

Statistical analysis

Results are presented as means ± SE for comparison of different experimental animal groups and control ones, the Student's t-test was used and the results were calculated by using origin program (version 6). Significance difference between control and treated groups n= 8.

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