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Possible ameliorative effect of curcumin and levothyroxine on potassium dichromate-induced hypothyroidism in male albino rats

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ABSTRACT

Potassium dichromate is a toxic heavy metal that contains chromium and causes hypothyroidism. The study aimed to evaluate the effectiveness of curcumin and levothyroxine in preventing potassium dichromate-induced hypothyroidism in adult male albino rats. Fifty six adult male albino rats were splited into eight groups (n=7/each). Group 1 (G1) was used as an untreated control group. G2 was administered corn oil (0.4 ml/rat). G3 was given curcumin (100 mg/kg/ b.wt). G4 was administered levothyroxine (20 µg/kg/ b.wt). From G2 to G4, rats were orally administered for 4 weeks. G5, G6, G7, and G8 were injected with potassium dichromate (2 mg/kg/b.wt) intraperitoneally for two weeks. Then, G6, G7, and G8 were received curcumin, levothyroxine, and curcumin/levothyroxine as in G3, and G4, respectively. The thyroid stimulating hormones (TSH), triiodothyronine (T3), thyroxine (T4), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and myeloperoxidase (MPO) were evaluated in serum. Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were measured in the thyroid tissue. Thyroid tissues were taken for histological examination. The results showed that in G5, a significant increase in TSH, and a decrease in T3 and T4 levels were reported when compared to G1. TNF-α, IL-6 and MPO have also significantly increased in G5. Furthermore, there was a significant decrease in GSH content, SOD, and CAT activities, and a significant increase in MDA level in G5 thyroid tissues. The histological examinations of thyroid tissues confirmed these results. The combination of curcumin and levothyroxine (G8) achieved the highest recovery from potassium dichromate (G5) toxic effects.

Keywords: Curcumin, Hypothyroidism, Levothyroxine, Oxidative stress markers, Potassium dichromate, Thyroid hormones

1. Introduction

Hypothyroidism is a chronic pathological condition associated with deficiency of thyroid hormones (T3 and T4). The thyroid gland's poor production of thyroid hormone causes primary hypothyroidism, the pituitary gland's inadequate secretion of thyroid stimulating hormones (TSH) causes secondary hypothyroidism, and the hypothalamus's lack of thyrotropin releasing hormones (TRH) causes tertiary hypothyroidism. Hypertension, dyslipidemia, insulin-insensitivity, infertility, cardiomyopathy, anemia, and neuromuscular dysfunction are all consequences of untreated hypothyroidism (Vigneshwar et al., 2021). Hypothyroidism can have serious side effects, including cardiovascular illness, infertility, musculoskeletal and neurological issues, and even death, if left untreated or treated insufficiently (Dhara et al., 2024). Thyroxine (T4) and triiodothyronine (T3) are the two primary thyroid hormones secreted by the thyroid gland, which is a metabolically active

endocrine gland. These two hormones influence nearly all physiological processes and are necessary for cellular metabolism, (Kar et al., 2022). Thyroid hormones are crucial for body metabolism, and improper secretion can lead to physiological consequences and regulate overall metabolism (Li and Lin, 2021). The production of thyroid hormones by thyroid follicular cells is essential for healthy growth, development, neuronal differentiation, and metabolism. The hypothalamic-pituitary-thyroid axis is a self-regulating circuit that includes the thyroid, anterior pituitary, and hypothalamus. T3 and T4 hormones are synthesized when TRH, which is generated by the hypothalamus, stimulates the release of TSH from the anterior pituitary gland (Kan and Kucukkurt 2022). T4 and T3 regulate body functions, but metabolic dysfunctions linked to thyroid diseases raise the chance of acquiring further abnormalities such as depression and anxiety disorders. It is estimated that 200 million people worldwide suffer from thyroid disorders (Panda et al., 2021).

Chromium (Cr), which is abundant in the environment, is poisonous and has emerged as a hazard to human society. Depending on the chemical form, exposure period, and dose, chromium toxicity varies (Salama et al., 2021). Hexavalent-Cr is categorized as a group I occupational carcinogenic substance in the study published by the International Agency for Research on Cancer (IARC) (Loomis et al., 2018). Approximately half a million industrial workers globally are exposed to Cr at work. Additionally, hexavalent-Cr contaminated water is a worldwide problem since it is the main way that the general public gets exposed to chromium. A soluble hexavalent-Cr compound, potassium dichromate ($K_2Cr_2O_7$) has wide use in a variety of industries (ElBakry and Tawfik, 2014). Potassium dichromate, a strong oxidizing toxic heavy metal containing Cr-VI, is used in paints, magnetic tape manufacturing, leather tanning, and as a catalyst for the production of hydrocarbons. When exposed to Cr -VI, which is widely present in the environment, causing carcinogenic and mutagenic effects in both humans and animals (Sedik et al., 2023). Potassium dichromate causes oxidative tissue damage because it is metabolically reduced to trivalent-Cr (Arivarasu et al., 2008). The sulphate anion transport system in the cell membrane facilitates the easy penetration of hexavalent-Cr, which is then reduced to other lower oxidation states and accumulates in different organs, causing a multiplex of reactive oxygen species (ROS) and organ damage (Barhoma, 2018). An imbalance between antioxidant and free radical indicators in the body is known as oxidative stress (Khazdair et al., 2018). Free radicals cause oxidative stress disorders, which cause lipids, proteins, and nucleic acids to oxidize (Amidi et al., 2012). The prognosis of the majority of inflammatory disorders is influenced by ROS, which are significant signal molecules. Endothelial dysfunction and tissue damage are caused by ROS generated by polymorphonuclear neutrophils, which are an important part of immunological defense in the inflammatory region (Mittal et al., 2014). Cytokines are soluble, low molecular weight proteins that facilitate communication and contact between cells. These proteins have a role in controlling tissue repair, inflammation, and immune system reactions. Tumor necrosis factor-alpha (TNF-α) is the primary proinflammatory cytokine which triggers the inflammatory response. It also has the ability to trigger other cytokines, including interleukin-6 (IL-6) (Turkmen et al., 2022).

One popular medication used to treat hypothyroidism is levothyroxine sodium by replacing thyroid hormones. Levothyroxine has many positive benefits, but it can also have negative ones, such as changes in behavior, heat intolerance, tachycardia, irregular breathing, restlessness, sleeplessness, palpitations, abdominal pains, and weight loss (Vigneshwar et al., 2021). The use of natural herbal products as supplemental or preventative medicines is growing in popularity as a way to combat the negative effects of medications used to treat diseases (Avci et al., 2022). Novel compounds come from natural products so natural source-based medications have significantly improved human health (Wu et al., 2018). Turmeric, rich in bioactive compounds, including curcumin, a potent polyphenol with anti-inflammatory properties, has been extensively researched for its impact on inflammatory and oxidative

balance (Dehzad et al., 2023). The natural polyphenol compound curcumin, which is extracted from the rhizomes of the turmeric plant, has numerous curative properties and gained popularity in biomedical fields due to its antioxidant, anticancer, antibacterial, anti-inflammatory, antiviral, neuroprotective, cardioprotective, and radioprotective activities (Pontes-Quero et al., 2021). Although hormone therapy may be effective in treating hypothyroidism, it also has a number of negative side effects. Therefore, the aim of this study was to evaluate the ability of the natural treatment curcumin and levothyroxine to treat rats' hypothyroidism induced by potassium dichromate.

Materials and methods

Chemicals

Potassium dichromate and curcumin (catalogue numbers 309176 and C1386, respectively) were purchased from Sigma-Aldrich Chemical Company in Cairo, Egypt.

Drug preparation

Eltroxin was obtained as tablets from Glaxo Smith Kline Drug Company. Each tablet contains levothyroxine (50 µg), which dissolved in distilled water and administered orally once daily for four weeks at a dose of 20 µg/kg b.wt/day (Farag et al., 2023).

Animal treatments

Fifty-six adult male albino rats were used in the study (12 weeks old, weighing 150- 160 g); they were acclimated for two weeks. Rats were purchased from Cairo's Egyptian Organization for Biological Products and Vaccines and housed in breeding cages. Rats were fed a standard diet (Egyptian Company of Oils and Soap, Kafr-El Zayat, Egypt) and water. The present research was given approval by the Committee of the Scientific Research Ethics of Faculty of Science, Damanhour University under Code No. DMU-SCI-CSRE- 24-08-03.

Rats were grouped into eight groups (seven rats for each group).

Group 1 (G1); Rats were used as control during the experiment. They were fed a regular diet and not given any treatment. G2 (corn oil group): Rats were given 0.4 ml corn oil orally by gastric gavage every day for 4 weeks. G3 (curcumin group): Rats were given 100 mg/kg b.wt/day of curcumin dissolved in corn oil orally daily for 4 weeks (Papiez et al., 2008).

G4 (levothyroxine group): Rats were administered orally levothyroxine at the dose of 20 µg/kg b.wt/day for 4 weeks (Farag et al., 2023). G5 (potassium dichromate group): Rats were injected potassium dichromate intraprotenially (i.p.) at a dose of 2 mg/kg/ b.wt, for 2 weeks, to induce hypothyroidism (El Bakry and Tawfik, 2014). G6 (potassium dichromate/ curcumin group): After receiving an injection of potassium dichromate for 2 weeks, rats were given curcumin as in G3 for an additional 4 weeks. G7 (potassium dichromate/ levothyroxine group): After receiving an injection of potassium dichromate for two weeks, rats received the levothyroxine as G4 for 4 weeks. G8 (potassium dichromate/curcumin/levothyroxine group): After receiving an injection of potassium dichromate for 2 weeks, rats was co-administering curcumin and levothyroxine as in G3 and G4, simultaneously for 4 weeks.

Collecting samples

After an overnight fast, the experiment involved rats being euthanized by using sodium pentobarbital i.p. (60 mg/kg) (Pourghasem et al., 2014) and investigated to a complete necropsy. Each rat's portal vein was used for gathering blood samples, which were then placed in non-heparinized, dry, and clean glass test tubes. Centrifugation of the specimens at 1922 g for 15 minutes at 4^{o}C was used to separate the serum. The thyroid gland removed by making a mid-line incision in the neck, identifying sternohyoid and sternomastoid muscles, and the muscles were separated to see the trachea. The thyroid glands were visible as two small reddish oval masses on each side of the trachea. The glands gently dissected to avoid its injury (Hadie et al., 2013). The thyroid glands were removed from the dissected rats and separated into two equal portions. Ice-cold saline solution was used to wash each portion. The first thyroid gland portion was preserved in 10% formalin for histological examination, while the second thyroid gland portion and the serum samples were kept at –80°C until they were needed for biochemical analyses.

Determination of thyroid hormones

Serum T3 and T4 levels were measured by using rat ELISA kits from Sigma-Aldrich Co. (St. Louis, MO, USA) with catalog numbers: SE120091 and SE120090, respectively. Serum TSH concentration in serum was measured by using rat ELISA kit from Cusabio Co. (Fannin, Houaton, TX, USA) with catalog number: CSB-E05115r.

Estimation of proinflammatory cytokines

Determination of TNF-α and MPO were performed by using rat ELISA kits from ELK Biotechnology Co. (Denver, CO, USA) with catalog number: ELK1396 and ELK10309, respectively. IL- 6 was determined by using Rat EKISA kit from Bioassay Technology Laboratory Co. (Yangpu, Shanghai, China) with catalog number: E0135Ra.

Preparation of thyroid gland tissue homogenate

The tissue homogenate of thyroid gland was prepared. To obtain 10% (w/v) homogenates, one gram of tissue was cut into small pieces and submerged in ice-cold 0.1M phosphate buffer saline (PBS, pH 7.4) one gram of tissue was sliced into small pieces and immersed in ice-cold 0.1M PBS (pH 7.4). Then the homogenates were centrifuged at 4ºC for 15 minutes at 1922 g, and the supernatants were separated and stored at -80ºC until use.

Estimation of thyroid tissues antioxidant parameters

Thyroid tissue antioxidant parameters were determined using the resulting supernatants: The commercial kits Biodiagnostic, Co. (Dokki, Egypt) with catalog number: GR 25 11 was used to measure reduced glutathione (GSH) at 412 nm, following a reaction involving the transformation of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to 5′-Thionitrobenzoate (TNB) (Beutler, 1963); the enzymes superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, E.C. 1.11.1.6) were measured using the Biodiagnostic kit (catalog numbers: SD 2521 and SD 2517, respectively). The Biodiagnostic kit (catalog number: MD-2529) was used to estimate malondialdehyde (MDA) levels by reacting with thiobarbituric acid (TBA) for 30 minutes at 95°C in acidic media, resulting in a pink product at 534 nm (Ohkawa et al., 1979).

Histopathological examination

The thyroid gland was processed for histopathological study using hematoxylin and eosin (Hand E) stain (Kiernan, 2015). Thyroid glands were removed from dissected rats, fixed in 10% formalin solution for 24-48 hours, and processed to form paraffin wax blocks. Paraffin blocks were prepared for sectioning at 5 μm thickness by rotary microtome and examined under light microscope.

Statistical analysis

The data was statistically analyzed using the Statistical Package for Social Sciences (SPSS software) version 25. The results were displayed as mean ± standard deviations (SD) and evaluated using the one-way ANOVA (Tukey) test for multiple comparisons. Statistical significance was considered as a p value ≤ 0.05.

3. Results

TSH, T3 and T4 in G2 and G3 showed near values to control rats, while rats administered levothyroxine (G4) showed significant decrease ($p< 0.05$) in TSH concentration and significant increase ($p< 0.05$) in T3 and T4 concentrations when compared to control group (G1). The data obtained from potassium dichromate induced hypothyroidism rats showed highly significant increase ($p< 0.05$) in TSH concentration that reached to about 1.9 fold and significant decrease ($p < 0.05$) in T3 and T4 concentrations in rats injected with potassium dichromate (G5) if compared with control group. Additionally, G6, G7, and G8 showed a significant decrease ($p< 0.05$) in TSH concentration compared to G5, while T3 and T4 concentrations increased significantly ($p< 0.05$) in these groups compared to G5. Furthermore, there is significant decrease ($p< 0.05$) in TSH concentration after treatment with curcumin (G6) and levothyroxine (G7) indicated that mild improvement in thyroid hormones concentrations if compared with G1 and G5. Treatment with co-administration of curcumin and levothyroxine showed a high degree of improvement in thyroid hormones concentrations which reached to normal values especially in G8 as shown in Fig. 1.

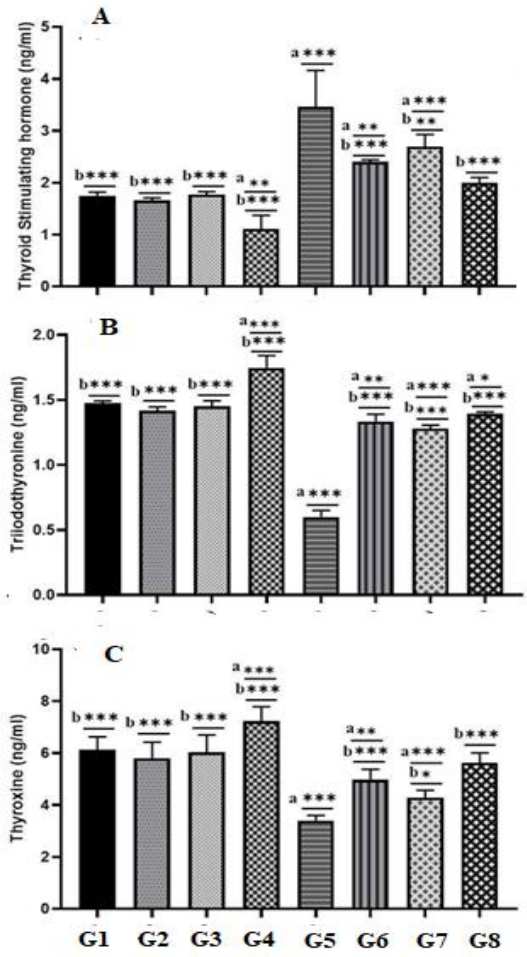

Fig. 1. The concentrations of TSH (A), T3 (B), and T4 (C) of all experimental groups. G1: control group; G2: Corn oil-group; G3: Curcumin group; G4: Levothyroxin group; G5: Potassium dichromate group; G6: Potassium dichromate/ curcumin group; G7: Potassium dichromate/levothyroxine group; G8: Potassium dichromate/curcumin/levothyroxine group. Results are shown as mean ± SD. (***), (**), and (*) the levels of significance. (a): significant at $p \leq 0.05$ in comparison with control group G1. (b): significant at $p \leq 0.05$ in comparison with potassium dichromate group G5.

Data obtained from Fig. 2 showed the changes in serum TNF-α, IL- 6, and MPO concentrations in potassium dichromate group (G5) were highly significant increase ($p < 0.05$) reached to about 1.49, 1.59, and 2.17 folds, respectively, if compared with control group. Also, proinflammatory cytokines significantly decrease ($p < 0.05$) in treated groups G6, G7, and G8 as compared to G5 and G1. The results obtained from this study showed antioxidant parameters (GSH, SOD, and CAT) and MDA revealed no significant change ($p > 0.05$) between the G2 and G3 groups compared to the control group. The results indicated a slight, statistically insignificant reduction ($p > 0.05$) in GSH content, and SOD and CAT activities in G4, with an insignificant increase ($p > 0.05$) in MDA level in comparison with the control group. However, the results from G5 revealed an incredible significant decrease ($p < 0.05$) in GSH content, and SOD and CAT activities and highly significant increase ($p < 0.05$) in MDA level when compared to the control group. Furthermore, there is significant increase ($p < 0.05$) in GSH content, and SOD and CAT activities in G6, G7 and G8 when compared to G5. Additionally, compared to G5, the MDA level in G6, G7, and G8 significantly decrease ($p < 0.05$) as shown in (Fig. 3).

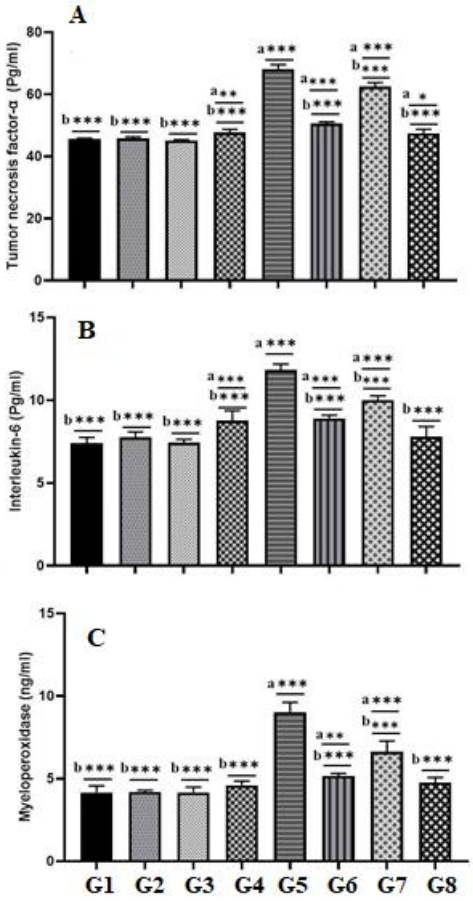

Fig. 2. The levels of TNF-α (A), IL-6 (B), and MPO (C) of all experimental groups. G1: control group; G2: Corn oil-group; G3: Curcumin group; G4: Levothyroxin group; G5: Potassium dichromate group; G6: Potassium dichromate/ curcumin group; G7: Potassium dichromate/levothyroxine group; G8: Potassium dichromate/curcumin/levothyroxine group.. (***), (*), and (*) the levels of significance. (a): significant at $p \leq 0.05$ of corresponding group in comparison with G1. (b): significant at $p \leq 0.05$ of corresponding group in comparison with potassium dichromate G5.

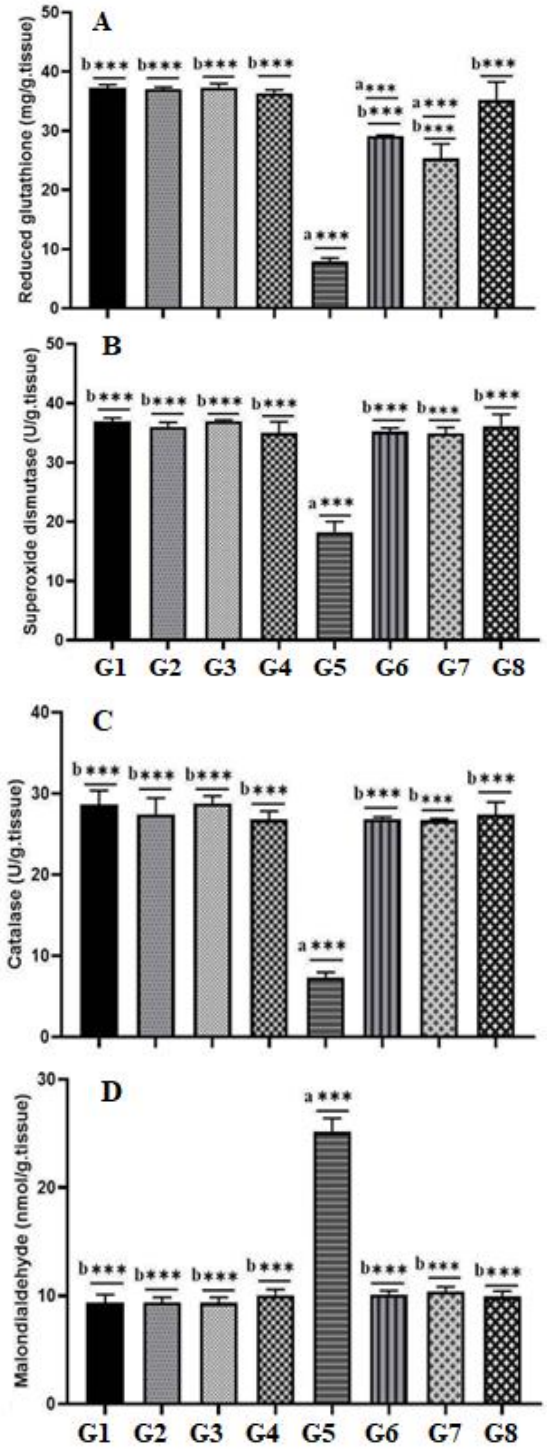

Fig. 3. The content of GSH (A), the activities of SOD (B), CAT(C), and the level of MDA (D) of all experimental groups. G1: control group; G2: Corn oil-group; G3: Curcumin group; G4: Levothyroxin group; G5: Potassium dichromate group; G6: Potassium dichromate/ curcumin group; G7: Potassium dichromate/levothyroxine group; G8: Potassium dichromate/curcumin/levothyroxine group. (***), (**) and (*) the levels of significance. (a): significant at $p \leq 0.05$ in comparison with G1. (b): significant at $p \leq 0.05$ of corresponding group in comparison with G5.

Histological examinations

Microscopic examination of thyroid gland sections of control and corn oil groups stained with haematoxylin and eosin revealed normal architecture, varying sizes, and acidophilic colloid filling in follicular lumens. Each follicle had a single layer of cubical cells with spherical nuclei (Fig. 4 A and B). The group treated with curcumin showed normal thyroid follicular histological structure when compared to the control group (Fig. 4 C). The levothyroxine treated group revealed normal thyroid follicle structure, but some had flatted follicular cells with flatted nuclei and acidophilic colloid in their follicular lumens if compared to G1 (Fig. 4 D). On the opposite side, the potassium dichromate group induced hypothyroidism and revealed disrupted thyroid follicle walls and loss of regular architecture. Most of the thyroid follicles were damaged and fused. However, in their lumen there are desquamated epithelial cells. In addition, the follicular cells were vacuolated cytoplasm and dark rounded (pyknotic) nuclei when compared to G1 (Fig. 5 A). The potassium dichromate/curcumin treated group revealed nearly normal thyroid follicles lined with spherically nucleated follicular cells and the follicular lumens filled with acidophilic colloid. A few of follicular cells exhibit vacuolated cytoplasm with dark rounded pyknotic nuclei if compared to G5 (Fig. 5 B). The potassium dichromate/ levothyroxine treated group showed mild improvements in thyroid follicles if compared to G5 (Fig. 5 C). The thyroid gland sections in potassium dichromate/curcumin/levothyroxine treated group revealed marked degree of improvements in thyroid gland architecture if compared to G5 (Fig. 5 D).

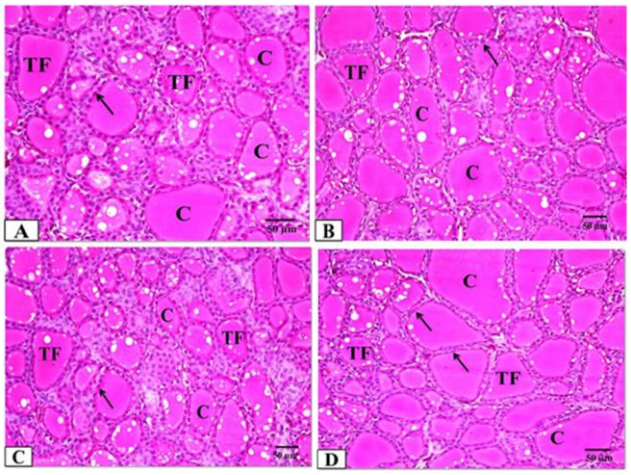

Fig. 4. Photomicrographs of thyroid gland sections were obtained, stained with haematoxylin and eosin control group (A) and corn oil group (B), revealing normal architecture of thyroid follicles (TF) appeared with variable sizes and the follicular lumens filled with acidophilic colloid (C). Each follicle had a single layer of cubical cells with spherical nuclei (arrows). Curcumin treated group (C), revealed normal histological structure of thyroid follicles (TF). Levothyroxine treated group (D) displayed the glandular structure of most thyroid follicles, but some thyroid follicles (TF) had a single layer of flattened follicular cells with flattened nuclei (arrow) and follicular lumens filled with acidophilic colloid (C). (H & E, x400).

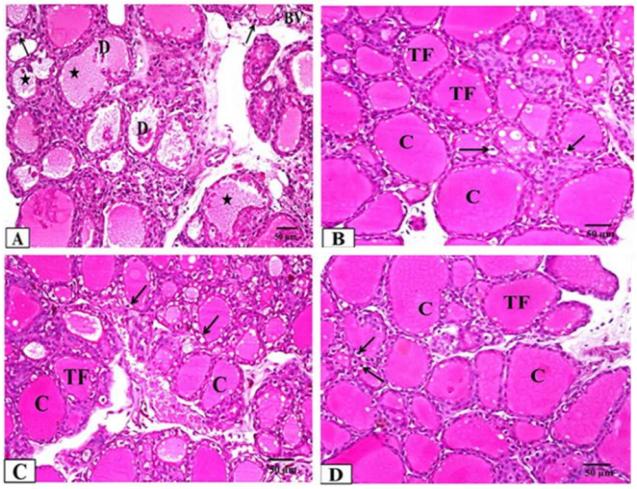

Fig. 5. Photomicrographs of thyroid gland sections. Potassium dichromate group (A), illustrating thyroid follicle walls were damaged and lost their normal architecture,in lumen of most thyroid follicles fused (star), the epithelial cells (D) were observed. Congested blood vessels (BV) and follicular cells with vacuolated cytoplasm and dark rounded (pyknotic) nuclei (arrow) were observed. Potassium dichromate/curcumin treated group (B) showing nearly normal thyroid follicles (TF) lined with spherically nucleated follicular cells and the follicular lumens filled with acidophilic colloid (C). A few of the follicular cells exhibit vacuolated cytoplasm and dark rounded (pyknotic) nuclei (arrow). Potassium dichromate/levothyroxine treated group (C) illustrating mild improvements in thyroid follicles (TF) and the follicular lumens filled with acidophilic colloid (C), with vacuolated cytoplasm and pyknotic (arrow). Potassium dichromate/curcumin/levothyroxine treated group (D) revealed marked degree of improvements in thyroid follicles (TF) and the follicular lumens filled with acidophilic colloid (C). (H & E, x400).

Discussion

As one of the hexavalent Cr compounds, potassium dichromate is regarded as a common inorganic chemical reagent with immunotoxin, carcinogenic, and genotoxic properties. Through oxidative stress, it causes toxicity to the thyroid gland (Belal et al., 2023). Thyroid hormone replacement therapy is currently used extensively to treat hypothyroidism, although it has a number of negative side effects, including heat intolerance, anxiety, headache, chest pain, nausea, elevated heart rate, and weak bones (Balkrishna et al., 2024). This clearly explains why curcumin, a strong antioxidant utilized in this study, was able to decrease the incredible increase in oxidative stress and inflammatory markers. Curcumin, a natural phenolic compound, has significant disease treatment potential because of its anti-inflammatory and antioxidant qualities. It controls inflammatory signaling pathways and reduces inflammatory mediators' production, while also acting as a free radical scavenger (El Kazzaz and Shehab, 2024). Thyroid hormones are essential for cell proliferation, differentiation, and metabolism regulation. Inhibition in their synthesis can cause health issues so; curcumin is turmeric polyphenol which stimulates the thyroid gland in rats (Papież, 2023). In this research, we investigated curcumin's preventive properties against potassium dichromate motivated hypothyroidism, oxidative stress, inflammation, and histopathological alterations in thyroid gland.

The result obtained from this study showed that rats administered with levothyroxine (G4) showed significant decrease in TSH and significant increase in T3 and T4 if compared with control group (G1). The alteration in hormonal concentration in G4 suggested a single layer of flattened follicular cells, which displayed flattened nuclei, surrounded the thyroid follicles, and the follicular lumens were filled with acidophilic colloid. Our results were confirmed by Hussein et al. (2022) who reported that rats orally administered with L-thyroxine (levothyroxine) at different doses of (50, 100 and 200 µg/kg bwt) for one, two and three weeks, respectively showed significant decrease in serum TSH and increase in T3 and

T4 concentrations. The thyroid gland, responsible for secreting two main thyroid hormones thyroxine and triiodothyronine which plays a crucial role in cellular metabolism and body functions, so hypothyroidism being a common condition caused a lack of thyroid hormones (Kar et al., 2022).

Serum TSH, T3, and T4 measurements are the most reliable methods for identifying hypothyroidism; a rise in TSH and a fall in T4 and T3 levels are considered indicators of hypothyroidism (Ashraf et al., 2022). Potassium dichromate induced hypothyroidism through biochemical and histological alterations in thyroid gland. G5 induced hypothyroidism in rats caused highly significant increase in TSH level reached to about 1.9 fold and significant decrease in T3 and T4 in rat if compared with normal rat. In agreement with the present results, Aboul-Fotouh et al. (2018) who confirmed that potassium dichromate-induced hypothyroidism results in decreased levels of T3 and T4, with increased TSH, related to the transformation of potassium dichromate to its trivalent form which increases oxidative stress and reactive oxygen species, there by affecting follicular cells' thyroid hormone production so, T3 and T4 decreased as a result of the active combination of Cr and globulins, which preventing the proteolysis of thyroglobulin. Similar findings were reported by Mohamed and Abd El-Twab (2016) who attributed the decreased serum free triiodothyonine (FT3), free thyroxine (FT4) and significant increase in TSH levels in G5 rats. Only the thyroid gland expresses the thyroperoxidase enzyme (TPO), which is responsible for the manufacture of thyroid hormones. To create the thyroid hormones, TPO oxidizes iodide to iodine, iodinates thyroglobulin, and links iodinated tyrosine residues. TPO must work properly for thyroid hormones to be generated; hence inhibiting this enzyme can significantly lower serum T4 levels (Ramhøj et al., 2021).

The histopathological investigations obtained from G5 showed thyroid follicle walls were damaged, and their typical architecture was lost. Most of the thyroid follicles were damaged and fused. However, in their lumen there are desquamated epithelial cells. In addition, Follicular cells were seen with vacuolated cytoplasm and dark rounded (pyknotic) nuclei when compared to G1. These results confirmed previous studies that demonstrated potassium dichromate caused structural alterations in thyroid follicular cells and disrupted the gland's normal architecture by causing thyroid follicles to join together and degenerate as well as desquamated cells in its lumen (ElBakry and Tawfik, 2014). In comparison to G5 (potassium dichromate), The present results indicated a considerable significant decrease in TSH and significant increase in T3 and T4 after treatment with curcumin (G6), levothyroxine (G7), and both curcumin and levothyroxine (G8). The greatest recovery of potassium dichromate poisoning on TSH, T3, and T4 is one of the best outcomes seen in G8. Additionally, there was a noticeable improvement in the thyroid gland's histological structure.

The GSH, SOD, and CAT are primary cellular antioxidant enzymes that are believed to be essential for life. Since these enzymes are in charge of detoxifying various ROS, assessing their activity often indicates the level of oxidation (Panda et al., 2021). These results showed that the induction of levothyroxine caused a slightly insignificant decrease in GSH content, and SOD and CAT activities and a slight increase in MDA level in G4 compared to G1. Additionally, our findings showed that G4 had slightly greater levels of TNF-α, IL-6 and MPO concentrations than in G1. Hypothyroidism can lead to increased oxidative stress and decreased antioxidant activity (Chainy and Sahoo, 2020). ROS production linked to chromium poisoning causes oxidative stress and disrupts the balance between oxidants and antioxidants (Velma and Tchounwou, 2013). SOD, CAT, and GSH are recognized to be essential for lowering free radicals and preserving antioxidant homeostasis in tissues (Ighodaro and Akinloye 2018). The results from G5 revealed a highly significant increase in MDA level and an incredible significant decrease in GSH content, and SOD and CAT activities when compared to G1. In addition, the results confirmed that there were highly significant increases in TNF-α and IL-6 and MPO levels in G5 when compared to G1. All of these findings correspond with a previous investigation by Mohamed and Abd El-Twab

(2016) who confirmed a significant reduction of GSH and SOD and disturbed serum TNF-α and IL-6 in G5 rats. The body's antioxidant defense system, in particular the decreased GSH, counteracts the overproduction of ROS. Because of its reducing properties and involvement in cellular metabolism, it is essential for preserving cell integrity (El-Guendouz et al., 2020).

The superoxide anion is converted to oxygen and hydrogen peroxide by the enzyme SOD and CAT subsequently breaks it down into water. However, glutathione peroxidase (GPx) prevents oxidative destruction of membrane lipids and catalyzes the conversion of GSH to oxidize glutathione (GSSG) from hydroperoxides, so protecting the cellular membrane from destruction. Furthermore, by inhibiting glutathione reductase (GR), chromium hexavalent delays the conversion of GSSG into GSH (Kalaiselvi et al., 2013). Cr binding to the enzyme's active site and/or excessive enzyme utilization in the scavenging of free radicals caused by hexavalent-Cr may be the cause of the suppression of antioxidant enzymes, which results in irreversible inhibition of enzyme function. These enzymes' decreased activity and the suppression of their protein production could be used as indicators of heavy metal poisoning (Karhib et al., 2022).

MDA is a persistent byproduct that is widely recognized as an indirect marker of elevated intracellular ROS production (Cherian et al., 2019). The propagation of free radical reactions brought on by lipid peroxidation may compromise the integrity of membranes and cause cell death (Bashandy et al., 2021). Potassium dichromate stimulates inflammation by increasing TNF-α, which in turn promotes the inflammatory immune response to tissue damage through overproduction of TNF-α and IL-1β (Mehany et al., 2013). Curcumin was regarded as a bifunctional antioxidant, promoting the production of antioxidant enzymes indirectly and scavenging ROS directly (García-Niño et al., 2013). Thus, it's possible that an antioxidative substance could aid in the treatment of hypothyroidism. After treatment, the GSH content, SOD and CAT activities increased significantly and the level of MDA decreased significantly in rats given curcumin (G6), levothyroxine (G7), and both curcumin and levothyroxine (G8), respectively, in comparison to G5. The results showed that all treated groups had a significant decrease in the TNF-α, IL-6, and MPO, especially in G8 rats. Recently, El Kazzaz and Shehab (2024) attributed the curcumin has the ability to reduce inflammation by inhibiting the production of IL-6 and TNF-α, which prevents the progression of disease. The ability of curcumin to interact with different inflammation-related molecular targets and prevent the synthesis of TNF-α, interleukins, and monocyte chemoattractant protein is believed to be the explanation of its anti-inflammatory properties. Aboul-Fotouh et al. (2018) recorded that curcumin had a protective effect against hypothyroidism and thyroid tissue damage induced by potassium dichromate. Similarly, Memarzia et al. (2021) observed one of *Curcuma longa*, Linn's primary phenolic active compounds, curcumin, has a number of pharmacological properties, including anti-inflammatory and antioxidant properties. Mokhtari-Zaer et al. (2020) reported that phenolic acids, polyphenols, and flavonoids as antioxidant compounds scavenge free radicals and prevent the oxidative mechanisms. Furthermore, levothyroxine assists in treating hypothyroidism, however there is some oxidative stress caused by it, as shown by the G4 data. The greatest results in G8 take into account the best recovery from potassium dichromate toxicity on the thyroid gland and produce near-normal levels of thyroid hormone concentration, antioxidant parameter, and proinflammatory cytokines. In conclusion, potassium dichromate is a highly toxic substance induces hypothyroidism. The most effective recovery of potassium dichromate's adverse effects on biochemical and histological investigations occurs when hypothyroidism is treated with a combination of curcumin and drug (levothyroxine) such as thyroid hormonal disturbance recovery, promoting antioxidant markers (GSH, SOD and CAT) and inhibition of TNF-α, IL-6 and MPO.

References

Aboul-Fotouh GI, El-Nour A, El-Din RK, Farag, E, Boughdady WAE, 2018. Histological study on the possible protective effect of curcumin on potassium dichromate induced hypothyroidism in adult male albino rats. Egypt. J. Histol. *41*(2), 220-235.

Amidi S, Mojab F, Moghaddam AB, Tabib K, Kobarfard F, 2012. A simple electrochemical method for the rapid estimation of antioxidant potentials of some selected medicinal plants. Iran. J Pharm Res: 11(1), 117.

Arivarasu NA, Fatima S, Mahmood R, 2008. Oral administration of potassium dichromate inhibits brush border membrane enzymes and alters antioxidant status of rat intestine. Arch. Toxicol.82(12), 951-958.

Ashraf H, Heydari M, Shams M, Zarshenas MM, Tavakoli A, Sayadi M, 2022. Efficacy of Ginger Supplementation in Relieving Persistent Hypothyroid Symptoms in Patients with Controlled Primary Hypothyroidism: A Pilot Randomized, Double-Blind, Placebo-Controlled Clinical Trial. Evid. Based Complement. Altern. Med. 1: 5456855.

Avci G, Ulutas E, Ozdemir V, Kivrak I, Bulbul A, 2022. The positive effect of black seed (Nigella sativa L.) essential oil on thyroid hormones in rats with hypothyroidism and hyperthyroidism. J. Food Biochem. *46*(4): e13801.

Balkrishna A, Paliwal R, Maity M, Varshney Y, Sinha S, Varshney A, 2024. Thyrogrit, supplemented with a sub-optimal dose of levothyroxine, restores thyroid function in rat model of propylthiouracil-induced hypothyroidism. Clin. phytosci, 10(1), 8.

Barhoma RA, 2018. The role of eugenol in the prevention of chromium-induced acute kidney injury in male albino rats. Alex. J. Med. 54(4), 711-715.

Bashandy SA, Ebaid H, Al-Tamimi J, Ahmed-Farid OAH, Omara EA, Alhazza IM, 2021. Melatonin Alleviated Potassium Dichromate-Induced Oxidative Stress and Reprotoxicity in Male Rats. Biomed Res. Int. 2021(1), 3565360.

Belal H, Rahman AA, Gouda SG, Hosny S, 2023. The Protective Effect of Selenium versus Ascorbic Acid on Potassium Dichromate-induced Thyroid Toxicity in Adult Male Albino Rats. J. Microsc. Ultrastruct. 1-10.

Beutler E, Duron O, Kelly BM, 1963. Improved method for the determination of blood glutathione. J Lab Clin Med.61: 882-888.

Chainy GB, Sahoo DK, 2020. Hormones and oxidative stress: an overview. Free Radic. Res.54: 1-26.

Cherian DA, Peter T, Narayanan A, Madhavan S. S, Achammada S, Vynat GP, 2019. Malondialdehyde as a marker of oxidative stress in periodontitis patients. J. Pharm. Bioallied. Sci. 11(Suppl 2), S297-S300.

Dehzad MJ, Ghalandari H, Nouri M, Askarpour M., 2023. Antioxidant and anti-inflammatory effects of curcumin/turmeric supplementation in adults: A GRADE-assessed systematic review and dose–response meta-analysis of randomized controlled trials. Cytokine.164:156144.

Dhara S, Chenchula SR, Chakraborty K, Valluru L, Surabhi G, 2024. Sulfated rhamnoglucan heteropolysaccharide of Spirulina platensis attenuates methimazole-induced hypothyroidism in rats. Algal Research. 78: 103409.

El Kazzaz NT, Shehab WI, 2024. Preventive Efficiency of Curcumin on Alveolar Bone of Hypothyroidism Rat Models with Induced Periodontitis (Histological, Ultrastructure and Micro-Elemental Study). Alex. Dental J. 1-9

El Bakry RH, Tawfik SM, 2014. Histological study of the effect of potassium dichromate on the thyroid follicular cells of adult male albino rat and the possible protective role of ascorbic acid (vitamin C). J. Microsc. Ultrastruct.,*2*(3), 137-150.

El-Guendouz S, Zizi S, Elamine Y, Lyoussi B, 2020. Preliminary screening of the possible protective effect of Moroccan propolis against chromium-induced nephrotoxicity in animal model. Vet. World, *13*(7), 1327.

Farag EA, Filobbos SA, Afifi NM, Mahmoud ST, Alghandour SM, 2023. Thyroxine restores hippocampal neurogenesis and synaptogenesis in a male rat model of carbimazole-induced hypothyroidism: a histological study. Beni-Suef Univ. J. Basic Appl. Sci.12(1): 57.

García-Niño WR, Tapia E, Zazueta C, Zatarain-Barrón ZL, Hernández-Pando R, Vega-García C. C, Pedraza-Chaverrí, J, 2013. Curcumin pretreatment prevents potassium dichromate-induced hepatotoxicity, oxidative stress, decreased respiratory complex I activity, and membrane permeability transition pore opening. Evid Based Complement Alternat Med 2013(1), 424692.

Hadie S, Abdul Manan H, Abdulla S, 2013. Thyroid gland resection in euthanized rat. A practical guide. Int Med J, 20(1), 1-4.

Hussein SA, ABDELAAL S, Marzouk M, Darweish M, Hussein AH, 2022. The potential ameliorative mechanisms of spirulina platensis or grape seed proanthocyanidin extract against thyroid disruptors and epigenetic micro-RNA derangements in L-thyroxine-induced hyperthyroidism in rats. Benha Vet. Med. J., *42*(2), 50-56.

Ighodaro OM, Akinloye OA, 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex. J. Med. 54(4), 287-293.

Kalaiselvi M, Gomathi, D., Ravikumar, G., Devaki, K., & Uma, C. (2013). Ameliorative effect of Ananus comosus peel on 7, 12 dimethylbenz (α) anthracene induced mammary carcinogenesis with reference to oxidative stress. J. Acute Disease. 2(1), 22-28.

Kan F, Kucukkurt, I, 2022. Investigation of the effect of boron on thyroid functions and biochemical parameters in hypothyroid induced rats. J. Biochem. Mol. Toxicol., *36*(11), e23186.

Kar A, Panda S, Singh M, Biswas S, 2022. Regulation of PTU-induced hypothyroidism in rats by caffeic acid primarily by activating thyrotropin receptors and by inhibiting oxidative stress. Phytomed. Plus. *2*(3): 100298.

Karhib MM, El-Sayed RA, Ghanem NF, El-Demerdash FM, 2022. Nephroprotective role of Echinacea purpurea against potassium dichromate-induced oxidative stress, inflammation, and apoptosis in rats. Environ. Toxicol, 37(9), 2324-2334.

Khazdair MR, Ghorani V, Alavinezhad A, Boskabady MH, 2018. Pharmacological effects of Zataria multiflora Boiss L. and its constituents focus on their anti-inflammatory, antioxidant, and immunomodulatory effects. Fundam Clin Pharmacol *32*(1), 26-50.

Kiernan J, 2015. *Histological and histochemical methods.* Scion publishing ltd.

Li ZG, Lin WT, 2021. Hypothyroidism and Obesity: Resistin May Inversely be Associated with Thyroid Function. Indian J. Pharm. Sci, *83*, 179-183.

Loomis D, Guha N, Hall AL, Straif K, 2018. Identifying occupational carcinogens: an update from IARC Monographs. Occup. Environ Med. 75(8): 593-603.

Mehany HA, Abo-youssef AM, Ahmed LA, Arafa ES, Abd El-Latif H A, 2013. Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. Beni Suef Univ J Basic Appl Sci. 2: 96-102.

Memarzia A, Khazdair MR, Behrouz S, Gholamnezhad Z, Jafarnezhad M, Saadat S, Boskabady MH, 2021. Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of Curcuma longa and curcumin, an updated and comprehensive review. BioFactors, 47(3), 311-350.

Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB, 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid. Redox Signal. 20(7): 1126-1167.

Mohamed HM, Abd El-Twab SM, 2016. Gallic acid attenuates chromium-induced thyroid dysfunction by modulating antioxidant status and inflammatorycytokines. Environ. Toxicol. Ph armacol. 48, 225-236.

Mokhtari-Zaer A, Norouzi F, Askari VR, Khazdair MR, Roshan NM, Boskabady M, Boskabady MH, 2020. The protective effect of Nigella sativa extract on lung inflammation and oxidative stress induced by lipopolysaccharide in rats. J. Ethnopharmacol. *253*, 112653.

Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., *95*(2), 351-358.

Panda S, Kar A, Singh M, Singh RK, Ganeshpurkar A, 2021. Syringic acid, a novel thyroid hormone receptor-β agonist, ameliorates propylthiouracil-induced thyroid toxicity in ratsJ. Biochem. Mol. Toxicol. *35*(8), e22814.

Papież MA, 2023. Curcumin exerts protective effects on the thyroid gland in propylthiouracil-treated rats. Folia Histochemica Et Cytobiologica, 61(3), 143-152.

Papiez MA, Kaja M, Gebarowska A, 2008. Age-dependent different action of curcumin in thyroid of rat. Folia Histochemica Et Cytobiologica, 46(2): 205-211.

Pontes-Quero GM, Benito-Garzón L, Cano JP, Aguilar MR, Vázquez-Lasa B, 2021. Amphiphilic polymeric nanoparticles

encapsulating curcumin: Antioxidant, anti-inflammatory and biocompatibility studies. Mater. Sci. Eng. C, *121*, 111793.

Pourghasem M, Nasiri E, Shafi H, 2014. Early renal histological changes in alloxan-induced diabetic rats. Int J Mol Cell Med. 3(1): 11.

Ramhøj L, Frädrich C, Svingen T, Scholze M, Wirth E K, Rijntjes E, Axelstad M, 2021. Testing for heterotopia formation in rats after developmental exposure to selected in vitro inhibitors of thyroperoxidaseEnviron. Pollut.*283:* 117135

Salama A, Fayed HM, Elgohary R, 2021. L-carnitine alleviated acute lung injuries induced by potassium dichromate in rats: involvement of Nrf2/HO-1 signaling pathway. Heliyon, 7(6), e07207.

Sedik AA, Hassan A, Salama A, 2023. Synergistic effect of arginine and Lactobacillus plantarum against potassium dichromate induced-acute liver and kidney injury in rats: Role of iNOS and TLR4/NF-κB signaling pathways. Iran J Basic Med Sci. 26(8), 941.

Turkmen R, Demirel HH, Akosman M, Fırat F, 2022. An investigation of the effect of chlorogenic acid on potassium dichromate-induced oxidative stress in rats. *Research Square*. 1-27.

Velma V, Tchounwou PB, 2013. Oxidative stress and DNA damage induced by chromium in liver and kidney of goldfish, Carassius auratus. Biomark. Insights, *8*, BMI-S11456.

Vigneshwar R, Arivalagan A, Palanivel M, 2021. Thyrogenic, hypolipidemic and antioxidant effects of Bacopa monnieri (Brahmi) on experimental hypothyroidism in rats. J Pharmacogn Phytochem, 10: 454-458.

Wu F, Li S, Zhang N, Huang W, Li X, Wang M, Han B, 2018. Hispidulin alleviates high-glucose-induced podocyte injury by regulating protective autophagy. Biomed. Pharmacother. 104, 307-314