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Role of *Tribulus terrestris* Against Cadmium-induced Toxicity on Thyroid Gland of Adult Male Albino Rats

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Abstract

Tribulus terrestris (Tt) is a herb distributed worldwide due to its beneficial claims on many diseases. This study aimed to study the role of Tt against Cadmium (Cd)-induced toxicity on thyroid gland of adult male albino rats. Twenty-four rats were subdivided into four groups; (Control group) without any treatment, (Tt + Cd group) with a daily oral dose of Tt (5 mg/ kg b. wt.) for eight weeks, from the beginning of the ninth week, they injected i.p. with 2 mg/kg of CdCl4 for 8 days, (Cd group) injected i.p. with 2 mg/kg of CdCl4 for 8 days, (Cd + Tt group) injected i.p. with 2 mg/kg of $CdCl_4$ for eight days, from the ninth day they were given a daily oral dose of Tt (5 mg/ kg b. wt.) for eight weeks. Results revealed that levels of T4 and TAC decreased while levels of TSH and MDA increased in Cd group resulted in follicular cells degeneration, increasing the amount of collagen fibers, and increased positive immunoreactivity of PCNA. Administration of Tt before Cd injection increased levels of T4 and TAC while decreased levels of TSH and MDA. administration of Tt after Cd injection didn't affect T4, TSH, MDA and TAC levels. The pre-treatment with Tt protects the thyroid tissue from those destructive changes by Cd while the post-treatment did not treat them. In conclusion, *Tribulus terrestris* is highly effective in shielding the thyroid gland from further damaging effects of Cd -induced oxidative stress when utilized as prophylactic antioxidant rather than treatment therapy.

KEYWORDS
Tribulus terrestris, Cadmium, Thyroid, T4, TSH, MDA, TAC, PCNA

INTRODUCTION

The thyroid gland is an endocrine gland situated in the front neck, beneath the larynx. It is a single gland with big lobes on the right and left that are joined by an isthmus in the middle (Eroschenko and Di Fiore, 2013). The thyroid gland is formed of numerous ball-shaped structures called follicles, and of interfollicular connective tissues containing blood capillaries. Each follicle is made up of many simple cuboidal epithelial follicular cells, a lumen encircled by the epithelial cells, and a few parafollicular cells that are found individually or in clusters in the basal region of the follicular epithelium (Mense and Boorman, 2018). Thyroid gland is concerned in producing, storing, and releasing of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). T4 is the quantitatively dominating hormone secreted by the thyroid gland, but T3 is physiologically more active and primarily results from peripheral deiodination of T4 (Rajab *et al*., 2017). Due to its complicated histological structure and functions, the thyroid gland is frequently the target of numerous endocrine toxins as cadmium (Jancic and Stosic, 2014).

Cadmium (Cd) is a well-known heavy metal present in pigments, batteries, steel, cement dust, mining, industry, and such as enduring of rocks, volcanic eruptions, and forest fires or in plant fertilizers (Dökmeci *et al*., 2009). It can be transmitted through water, soil and air. Also, the major sources of Cd are cigarette and diet as peanuts, spinach, soybeans, potatoes and sunflower seeds (Chung *et al*., 2019). It recognized as an endocrine disrupting chemical (Henson and Chedrese, 2004), such as metabolic syndrome (Lee and Kim, 2013), diabetes (Barregard *et al*., 2013), obesity (Tinkov *et al*., 2017), atherosclerosis (Fittipaldi *et al*., 2019), reproductive toxicity (Nasiadek *et al*., 2018), hypertension, chronic kidney disease, osteoporosis, and leukemia, as well as cancers of the kidney, lung, urinary bladder, breast, pancreas and prostate (Henson and Chedrese, 2004). Elevated levels of blood Cd may lead to elevated, decreased, or unchanged triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), or thyroid autoantibodies, depending on sex or doses (Buha *et al*., 2018). Cd leads to a decrease in antioxidant enzymes and increase oxidized free radicals causing infections that are directly proportional to the amount of Cd dose exposed. Since it does not have any vital function and cannot be degraded by living organisms, so it accumulates in among the various heavy metals because of its long biological half-life, low toxicity dosage, difficult to excrete out from the body and its ability to be stored in tissues (Barbier *et al*., 2005).

Tribulus terrestris L. is a well-known and widely cultivated species of the genus *Tribulus*. It is also known by its Arabic names, Al-Gutub, Qutiba, Hasak, or Ders El-Agouz. Common names include puncture vine, caltrop, goat head, bull's head, ground burr nut, and devil's thorn (Al-Ali *et al*., 2003; Kostova and Dinchev,

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2005). Throughout history, Tt has been used in folk medicine to treat diseases like impotence, rheumatism, oedema, hypertension, and kidney stones (Hammoda *et al*., 2013). it has been regarded as an aphrodisiac due to its beneficial claims on many diseases such as inflammations, urinary infections, ascites, and oedema (Gauthaman and Ganesan, 2008).Other studies showed that Tt recorded as an antioxidant and protective effect against diabetes and heart disease (Amin *et al*., 2006; Ojha *et al*., 2008). This plant is a possible powerful natural source of antioxidants and could be useful in scavenging free radicals (Zheleva-Dimitrova *et al*., 2012). This study aimed to investigate the effect of Cd on the thyroid gland, evaluate the role of Tt against these effects, and compare between Tt's prophylactic and therapeutic effects.

MATERIALS AND METHODS

Drug and Animals

Cadmium chloride purchased from C.P. Evans. Co, Egypt. *Tribulus terrestris* natural extract purchased from Elmo.natur, 604289 Leipzig, Germany. ELISA Kit for plasma thyroxine T4 estimation was purchased from SunLong Biotech Co. LTD, Hangzhou, China. ELISA Kit for plasma thyroid stimulating hormone (TSH) estimation was purchased from Cusabio Co., Houston, USA. Reagent kits for plasma levels of lipid peroxide Malondialdehyde (MDA) and total antioxidant capacity (TAC) estimation were obtained from Biodiagnostic, Giza, Egypt.

Twenty-four adult male albino rats (weight of 150–200 g) were used. One week prior to the experiment, the animals acclimatized for diet, kept in separate cages with free access to feed and drink water, standard laboratory conditions were maintained, controlled temperature 28 ~ 34 °C and relative humidity (50±10) %, 12 hours of the light-dark cycle were maintained.

Ethical approval

Under the National Institutes of Health criteria for the use of experimental animals; The research protocol used in the present study was reviewed and accepted by the medical ethics committee of Assiut University's Faculty of Medicine; Egypt, (IRB no:17300892).

Experimental Design

Animals were randomized and subdivided into four groups (6 rats/group):

Group 1 (Control group): without any treatment.

Group 2 (Tt + Cd): was given a daily oral dose of *Tribulus terrestris* (5 mg/ kg b.wt. dissolved in d.w) for eight weeks (Gauthaman and Ganesan, 2008). From the beginning of the ninth week, they injected intraperitoneally with 2 mg/kg of $CdCl_4$ (Olaniyan *et al*., 2021) for eight days.

Group 3 (Cd): injected intraperitoneally with 2 mg/kg of $CdCl_4$ for eight days (Olaniyan *et al*.,2021).

Group 4 (Cd +Tt): injected intraperitoneally with 2 mg/kg of $CdCl_4$ for eight days. From the ninth day they were given a daily oral dose of *Tribulus terrestris* (5 mg/ kg b. wt.) for eight weeks.

At the end of the experiment in each group, rats were anesthetized and immediately blood samples were collected from jugular vein. Then rats dissected to obtain the thyroid gland for histological and immunohistochemical studies. Blood samples collected in EDTA containing tubes, following centrifuging at 3000 rpm for 10 minutes, plasma was obtained and stored at -20 °C for estimation of T4, TSH, MDA and TAC.

Histological study

For histopathological examination, tiny thyroid gland sections were quickly obtained and fixed in 10 % neutral buffered formalin (pH 7.2); thyroid sections were routinely prepared using the paraffin-embedded method. Then they were washed and dehydrated in an ascending grade of ethanol solutions (70-100%) for water removing, cleared in xylene, and then embedded in wax. A rotatory microtome was used to slice paraffin blocks into 5 µm thickness and then deparaffinized (dewaxed) in xylene, and. The standard staining procedure of Hematoxylin and Eosin (for the histological examination of the thyroid), and Masson trichrome (for type I collagen fiber) stains were followed (Suvarna *et al*., 2018). A digital camera (Toup Tek ToupView, Copyrightc 2019, Version:x86, Compatible: Windows XP/Vista/7/8/10, China) was used for the examination and photography. A computer connected to a light microscope (Olympus CX31, Japan).

Immunohistochemical staining for proliferating cell nuclear antigen (PCNA)

Proliferating cell nuclear antigen (PCNA), the intranuclear polypeptide that is involved in DNA replication, excision and repair. Cell proliferation was associated with both its production and expression (Shivji *et al*., 1992). thyroid tissues were fixed in 10 % neutral buffered formalin (pH 7.2), paraffin-embedded tissues were sectioned, cleared, and rehydrated in a grade of ethanol solutions (100%–70 %) and submerged in water. Antigens were extracted by boiling the slides in 1 mM ethylenediaminetetraacetic acid (EDTA) for 10 min, emerging sections in 3 % H_2O_2 for 10 min. For one hour, each section stayed at room temperature in a blocking solution. The primary anti-PCNA IgG antibody was diluted in Trisbufferd saline then added for 24 h, followed by the secondary antibodies (1: 5000) for 2 h. After establishing the reaction with DAB for 2–3 min, sections were stained with hematoxylin for 2–5 min.

Statistical Analysis

The statistical significance of groups was determined by Student's t-test and one-way ANOVA ($P < 0.001$). All assays were performed in triplicate. Statistical analyses were performed using Prism software (version 8.0.1; GraphPad Software, Inc San Diego, CA, USA). Fiji/Image J software for graph image were used.

RESULTS

Estimation of T4, TSH, MDA and TAC levels

The effect of Tt administration on levels of plasma T4, TSH, MDA and TAC of rats injected with Cd is presented in Table 1. There was a significant decrease in the levels of T4 and TAC ($P < 0.001$) while there was a significant increase in the levels of TSH and MDA ($P < 0.001$) after Cd injection of rats compared to control group. Administration of Tt before Cd injection increased the levels of T4 ($P < 0.001$) and TAC ($P < 0.01$) while decreased the levels of TSH and MDA compared to Cd group ($P < 0.01$). However, administration of Tt after Cd injection didn't affect T4, TSH, MDA and TAC levels of rats compared to Cd group ($P > 0.05$).

Histopathological examination

Stained sections with H and E of thyroid gland in control group revealed active thyroid follicles of various sizes (Fig. 1a).

Table 1. Showing the effect of Tt administration on T4, TSH, MDA and TAC of rats injected with Cd.

Treatment	Control	Tt + Cd	Cd	Cd + Tt	P value
T4 (ug/dl)	4.246±0.04411[a]	3.226±0.20030[b]	1.874±0.1533[c]	1.702±0.1926[c]	0.082
TSH (µIU/ml)	0.9560±0.00812[a]	1.050±0.02191[b]	1.144±0.01364[c]	1.148±0.01497[c]	0.351
MDA (nmol/ml)	8.780±0.05831[a]	9.320±0.09695[b]	9.800±0.09487[c]	9.820±0.06633[c]	0.716
TAC (mM/L)	1.010±0.01304[a]	0.9300±0.01473[b]	0.8520±0.01530[c]	0.8040±0.005099[c]	0.255

T4: Thyroxine; TSH: Thyroid stimulating hormone; MDA: Malondialdehyde; TAC: Total antioxidant capacity.
Data are presented as Mean±SE. Values in the same row followed by different superscript (a,b,c) are significant (P < 0.05)

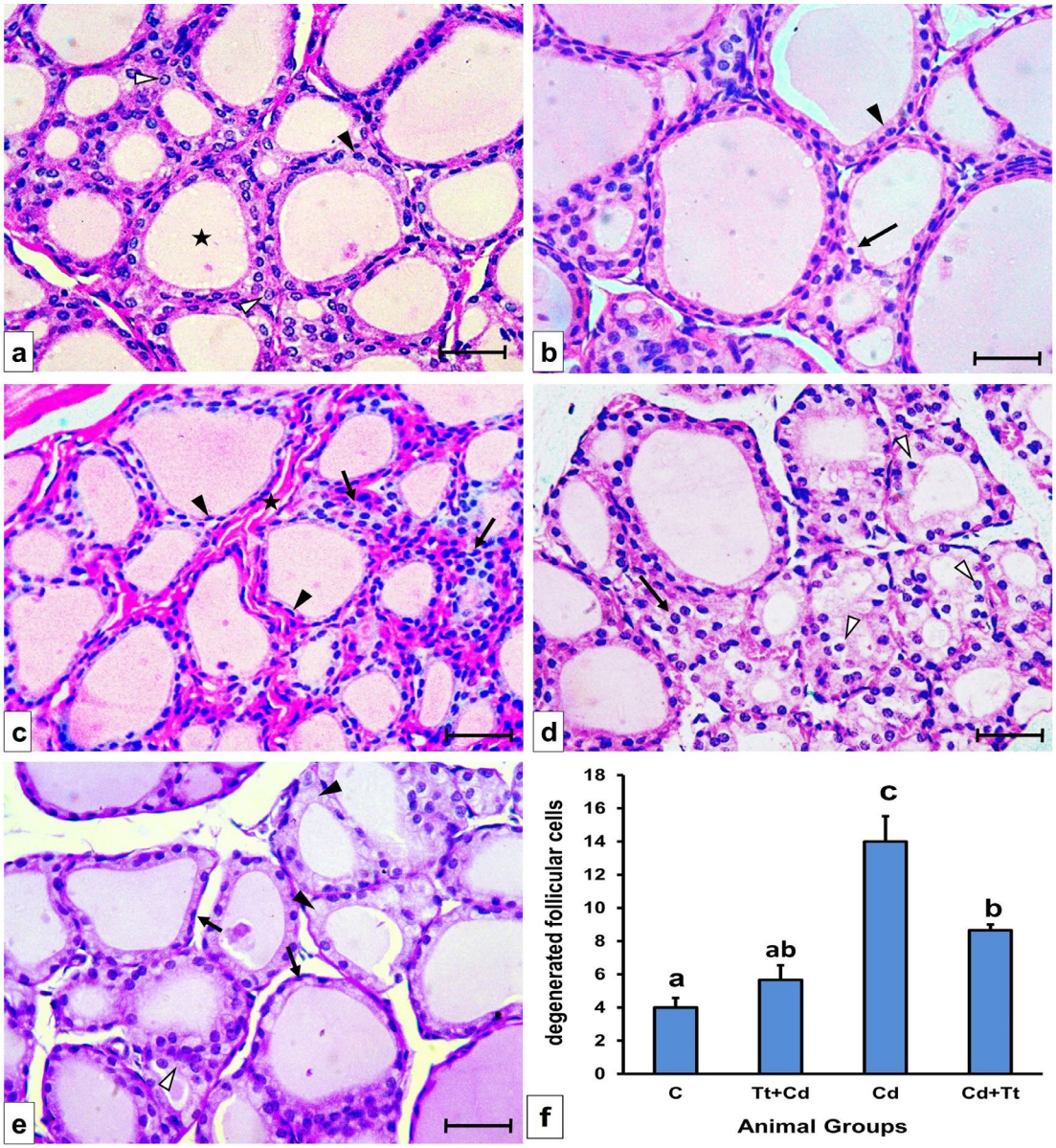

Fig. 1. Histopathological examination in different experimental groups: (a-e): Photomicrographs of thyroid gland sections stained by H&E, bar = 50µm. (a) In control group showing cuboidal follicular cells (▲), colloid (asterisk), and parafollicular cells (Δ). (b) In Tt + Cd group showing cuboidal follicular cells (▲) and few follicular cells with condensed nuclei and vacuolated cytoplasm (↑). (c & d) In Cd group showing flattened nuclei follicular cells (▲), follicular cells with condensed nuclei and vacuolated cytoplasm (Δ), acidophilic fibrous tissue (asterisk), and interfollicular cells with variable sizes and staining affinity (↑). (e) In Cd + Tt group showing karyolysis in follicular cells with flattened nuclei (▲), follicular cells (↑), and many cells in interfollicular tissue (Δ). (f) Number of degenerated follicular cells in the different experimental groups. Values in the column with unlike superscript letters are significantly different at (P < 0.001). Data represents mean±S.E.M.

Most follicles had cuboidal epithelium lining them and colloid in the lumen. The parafollicular cells on the basement membrane appeared as clear cells in the interfollicular spaces or between the follicular cells, but they do not reach the lumen. The interfollicular tissue was minimal and contained blood capillaries. Numerous thyroid follicles in the Tt + Cd group were active with cuboidal cell lining and deposited colloid in the lumen. A few follicular cells appeared with condensed nuclei and vacuolated cytoplasm (Fig. 1b). The interfollicular tissue included a few cells and blood capillaries. In Cd group, most of thyroid follicles were with flat squamous lining epithelium (Fig.1c). Some follicles had poorly defined basement membranes and damaged lining epithelium (Fig. 1d). This damage in the follicular cells was represented as condensed nuclei with vacuolated cytoplasm. The interfollicular tissue showed abundant acidophilic fibrous tissue. The presence of many cells in the interfollicular tissue with variable sizes and staining affinity was the clear finding in this group (Figs. 1c and d). In the Cd + Tt group, some follicles lined with flattened follicular cells whereas the others had necrotic cells with karyolysis (Fig. 1e). Many cells are seen in the interfollicular tissue. Counting

the degenerated follicular cells in thyroid follicles from all the experimental group represented in Figure 2f. There were non-significant differences present in the number of damaged follicular cells between control group (4.0±0.58) and Tt + Cd (5.67±0.88) one. Significant increase (P < 0.001) in the number of degenerated follicular cells in Cd group (14.0±1.52) and Cd + Tt group (8.67±0.33) when compared with those in control group.

Collagen fibers examination

The Masson's trichrome staining of the thyroid sections revealed the quantity and distribution of collagen fibres in all of the experimental groups. In control group, there was mild amount of collagen fibers (1.47 %) around the follicles and in the interfollicular tissue (Fig. 2a). A tiny amount of collagen fibers (4.55 %) was found in the Tt + Cd group, almost comparable to those in the control group (Fig. 2b). Massive collagen fibres distribution (22.55 %) is observed in the Cd group surrounding blood capillaries, follicles, and in the interfollicular tissue (Fig. 2c). Compared to the control group, this increase in collagen fibres was statistically significant (P < 0.001). In Cd + Tt group, moderate amount of collagen fibers was observed (11.65 %) in the interfollicular tissue and around the follicles (Fig. 2d). Comparing this amount to those in the control group, it increased significantly (P < 0.001).

Immunohistochemical evaluation of PNCA expression

In control group, there was minimal positive immunoreactivity of PCNA in the nuclei of the follicular lining cells and in the interfollicular tissue (Fig. 3a). Comparing the Tt + Cd group to the control group, there was a non-significant increase (2.51 %) in PCNA immunoreaction (Fig. 3b). Cd group had a remarkable significant (P < 0.001) increase in PCNA positive immunoreactivity (36.27 %) compared to control group (Fig. 3c). This increase was represented as dark brown staining of the nuclei in the interfollicular tissue and in the epithelial lining of the damaged follicles. In Cd + Tt group, moderate possitive immunoreaction of PCNA was present in the interfollicular tissue and in the interfollicular lining of the follicles. Comparing this reaction to that of the control group revealed a statistically significant increase (7.52 %) (Fig. 3d).

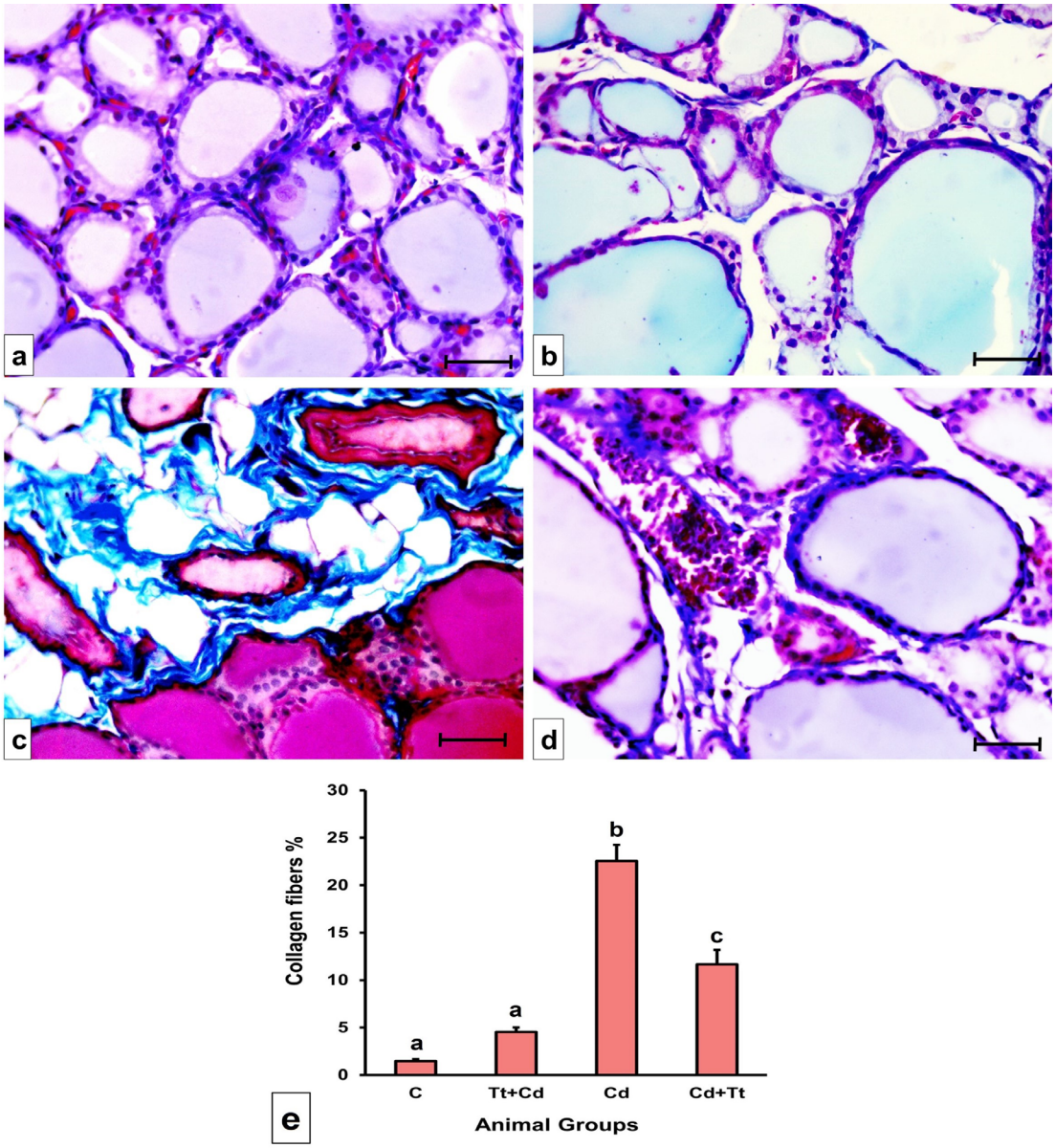

Fig. 2. Collagen fibers examination in the different experimental groups: (a-d): Photomicrographs of thyroid gland sections stained by Masson's trichrome stain, bar = 50µm. (a) In control group. (b) In Tt + Cd group showing mild amount of collagen fibers. (c) In Cd group showing massive collagen fibers distribution. (d) In Cd + Tt group showing moderate amount of collagen fibers. (e) Percentage of collagen fibers in the different experimental groups. Values in the column with unlike superscript letters are significantly different at (P < 0.001). Data represents mean±S.E.M.

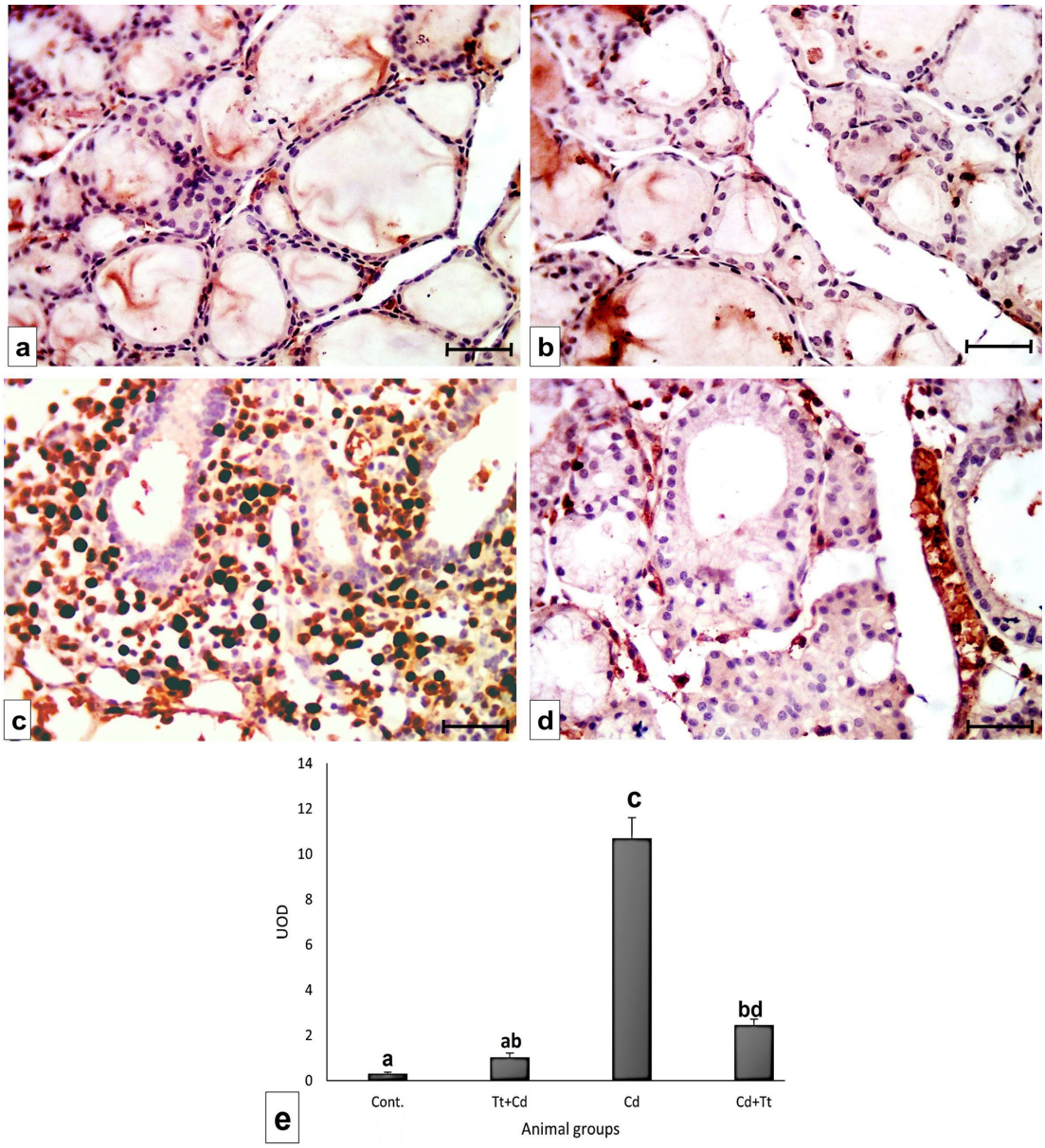

Fig. 3. Immunohistochemical evaluation of PCNA expression in the experimental groups: (a) In control group. (b) In Tt + Cd group showing mild positive immunoreactivity of PCNA. (c) In Cd group showing marked increase of PCNA positive immunoreactivity in the nuclei of the interfollicular tissue and in the epithelial lining of the damaged follicles. (d) in Cd + Tt group showing moderate PCNA possitive immunoreaction. Bars = 50µm (e) Densitometric levels of positive PCNA patches. Values in the column with unlike superscript letters are significantly different at ($P < 0.001$). Data represents mean±S.E.M.

DISCUSSION

The current study showed that injection of Cd significantly decreases TAC and increase MDA while administration of Tt + Cd significantly increases TAC and decrease MDA levels. That agrees with Olisekodiaka *et al.* (2012) who found a significant decrease in TAC in rats exposed to Cd that could be due to participation of the body's antioxidant system in combating to the increased free radical load resulting from Cd toxicity. Cd decreases antioxidant enzymes (Kumar *et al.*, 2019; Nemmiche, 2017) as it inhibits enablers that activate genes responsible for antioxidant enzymes production (Das and Al-Naemi, 2019). Also, Cd generates oxidative free radicals that are directly proportional to the Cd dose causing break down of cell components, affect cell calcium levels, stimulate apoptosis (Shagirtha and Miltonprabu, 2016), influencing cell physiology and growth (Ramirez and Gimenez, 2000; Lafuente *et al.*, 2003). Additionally, acute exposure to Cd causing multiple alterations in lymphocyte function includes reactive oxygen species (ROS) generation, DNA mutations and fragmentation leading to cell death by necrosis and apoptosis. It clearly interacts with multiple protein systems involved in modulating oxidative stress responses (Alkharashi *et al.*, 2017). In a previous study, Tt exerted a protective effect against testicular damage in rats exposed to Cd that revered to its antioxidant effect and stimulation of testosterone production from the Leydig cells (Rajendar *et al.*, 2011).

In this research, Cd injection significantly decreases T4 and increases TSH while administrations of Tt before Cd significantly induce T4 production and reduce TSH level. This is confirmed by Buha *et al.* (2018) who recorded that rat exposed to Cd showed decrease in T4 level. Cd seems to be the large single contributor to auto-immune thyroid disease (Gupta and Kar, 1999). It may be due to accumulation of Cd in thyroid gland causing oxidative stress, generation of ROS by mitochondria and dysfunction of the follicular cells reducing T4 synthesis and secretion (Buha *et al.*, 2018; Jancic and Stosic, 2014). Its precipitation in follicular epithelial cells causing deterioration of the rough-surfaced endoplasmatic reticulum and disappearance of Tg-secreting granules (the precursor of the thyroid hormones) was noted in follicular epithelial cells, which in turn may cause a significant decrease of the thyroid hormones release into circulation (Alkharashi *et al.*, 2017).

Cd produces oxidative stress in the endocrine organs, resulting in organ secretory abnormalities as in pancreatic beta-cells; Cd exposure leading to oxidative stress and mitochondrial dysfunction (Chang *et al.*, 2013). Experimental result showed that Cd at low environmental doses can disturb thyroid gland function (Buha *et al.*, 2018). It has been confirmed that Cd levels in thyroid

gland are three times higher in people living in Cd polluted areas than other in nonpolluted areas. It may be due to presence of cysteine-rich proteins which bind with Cd molecules converting into strong intracellular Cd detoxifier. Also, Cd binds to sulfhydryl molecules in thyroid gland as metallothioneins, glutathione slowing its excretion (Uetani *et al*., 2006; Klaassen *et al*., 2009). Other found that Cd combined with selenium atoms then excreted out from the body via bile, when selenium depleted by Cd causing reduction of 5 – deiodinase enzymes in kidneys and liver which is important in converting T4 to T3 resulting in hypothyroidism, also the decrease in selenium leads to depletion in gluthathione peroxidase (antioxidants), leading to increase level of hydrogen peroxide and reactive oxygen damaging thyroid gland (Ghosh and Bhattacharya, 1992).

In addition, several studies have shown that TSH increased following Cd exposure (Wade *et al*., 2002; Lafuente *et al*., 2003; Mohamed *et al*., 2015). Other studies approved that exposure to Cd showed abnormal hormonal thyroid function that evaluated by elevated TSH levels possibly because of positive feedback control mechanisms (Hammouda *et al*., 2008; Jurdziak *et al*., 2018). Blood Cd levels positively correlated to higher TSH and hypothyroid status (Silva *et al*., 2012). Also, it had been reported that there is a significantly decreased T4 levels in the group treated with a higher Cd level so both thyroglobulin and TSH mRNA were upregulated (Buha *et al*., 2018).

However, some research disagrees with that as a study on the effects of Cd and lead on the thyroid function of welders, showed no changes in T4, T3 and TSH (Sherif *et al*., 2017). While other showed an elevated blood Cd with decreased TSH levels, suggesting this inverse relationship between CD and TSH indicates overt thyroid disease due to Cd exposure (Meeker *et al*., 2009). These differences can be revealed to the route of Cd exposure (inhaled, ingested or absorbed), the dose and the duration of exposure (acute or chronic), affecting absorption rate and distribution of Cd, as well as the species and used protocols determine whether Cd will produce an increase, decrease or no effect on thyroid hormones plasma levels (Buha *et al*., 2018).

From histological point of view, the current study explained that cd administration to adult male rats resulted in many degenerative changes in the thyroid tissue. From these changes the degeneration of the follicular cells and poorly defined basement membranes. These findings supported by Ya *et al*. (2021), who claimed that structural thyroid damage caused by Cd, such as follicular distortion and epithelial cell hyperplasia, could serve as a sign of thyroid dysfunction.

According to Yang *et al*. (2021), oxidative stress brought on by Cd toxicity damages DNA in a variety of cell types, resulting in aberrant metabolism and structural damage such necrosis of follicular epithelial cells, hyperplasia, and a thinning of the thyroid cell membrane border. They further hypothesised that Cd would cause endoplasmic reticulum stress and activate the ERK1/2 pathway in thyroid follicular epithelial cells, mediating apoptosis. Sedky *et al*. (2017) added that Cd caused cytoplasmic vacuolation via inducing endoplasmic reticulum proliferation and mitochondrial enlargement. Additionally, apoptosis causes Cd -induced nucleus condensation.

The present study found that Cd injection resulted in an increase in the amount of collagen fibers among the thyroid tissue. This finding agrees with Veličkov *et al*. (2013) who stated that by causing mast cells to degranulate and release their cytokine tryptase, Cd caused fibrosis. This cytokine promotes fibroblast proliferation and collagen fibers formation. Additionally, they stated that selenium reduces when exposed to Cd. Through inflammatory response and excessive growth factor transforming stimulation, this deficit in selenium may cause fibrosis to become active.

The current study declared that Cd caused massive interfollicular cells proliferation. According to Waalkes (2000), Cd can activate some proto-oncogenes or genes linked to cell proliferation in both cells and animals, including c-myc and c-jun. He added that if there is a baseline number of cells with DNA damage caused by chemicals or spontaneous mutations, this activation may increase cell proliferation and, assuming colony growth of these damaged cells, colonisation. Cd could increase the population of cells with damaged DNA by inhibiting DNA repair.

In this study, pre-treatment with Tt to Cd administered rats protected the thyroid tissue to a great extent from subsequent damage caused by Cd. This was obvious through the histopathological observation, collagen fibers examination and, PCNA immunoreactivity administration.

The obtained findings concur with those of Rajendar *et al*. (2011), who claimed that Tt had a protective effect against testicular damage brought on by Cd. This protective effect appears to be mediated through antioxidant and metal chelator action, which inhibits the peroxidation of testicular tissue. Hammoda *et al*. (2013) explained that the antioxidant activity of Tt as measured by DPPH free radical scavenging activity revealed that the di-p-coumaroylquinic acid derivatives possess strong antioxidant activity and are therefore thought to be the main constituents contributing to the antioxidant effect of the plant. Research by Siddique *et al*. (2022), explored that in vitro free radical potential for the Tt extract was found to have a high level of free radical scavenging activity and good antioxidant properties. According to the study of Zhu *et al*. (2017), Tt exhibited potent antioxidant activity in a concentration-dependent manner as measured by the FRAP (Ferric reducing antioxidant power) assay, 2,2 di-(4-tert-octylphenol)-1-picrylhydrazyl (DPPH), H_2O_2, and superoxide scavenging activity.

In this study, oral administration of Tt extract after injection of Cd to male rats did not protect thyroid tissue from damage in most of the experiments. It's probable that either the significant tissue damage brought on by the Cd injection or the brief duration of the treatment in Tt plants is to blame.

CONCLUSION

From this study, we can conclude that Cd causes toxic effects on thyroid gland which are related to oxidative stress. the *Tribulus terrestris* plant is highly effective in shielding the tissues of the thyroid gland of male rats from further damaging effects of Cd when utilized as prophylactic antioxidant rather than treatment therapy.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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