Original Article

Possible Protective Effect of Grape Seed Extract and Curcumin on Triphenyltin Chloride Induced Thyroid Toxicity in Adult Albino Rats

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ABSTRACT

Introduction: Triphenyltin chloride (TPTC) is one of organotin compounds (OTs). It is present in multiple products as, disinfectants, pesticides, fungicidal wood preservatives and leather-processing facilities. OTs is considered one of the endocrine disruptors. Mammals are affected with these compounds through consumption of marine foods. **Aim of the work:** the aim was to study the possible protective effect of both grape seed extract (GSE) and curcumin on thyroid gland in the adult albino rats. **Material and method:** Fifty four adult albino rats were randomly divided in to 7groups: Group I (control) contained 18 rats is subdivided in to 3 subgroups; group Ia (negative) no treatment, group Ib, Ic (vehicle): rats received 0.5 ml distilled water, 0.5 ml corn oil respectively daily for 4 weeks. The other 36 rats are equally divided in to 6 groups (6 rats in each) received treatment daily for 4 weeks through oral gavage**.** Group II (GSE): at dose 150 mg/kg/day, group III (Curcumin): at dose 100 mg/kg/day, group IV (TPTC): at dose 10 mg/kg/day. There are group V (TPTC + GSE) group VI (TPTC+ Curcumin); group VII (TPTC + GSE + Curcumin). At the end of 4 weeks the rats were weighed, serum was obtained for thyroid hormons level, maliondialdehyde (MDA), glutathione peroxidase (GPx) enzyme. Rats were sacrificed, thyroids prepared for histopathological by hematoxylin and eosin (H&E) , immunohistochemical examination of Caspase-3 and Ki 67 by light microscope and morphometric analysis **Results:** TPTC adminstration resulted in significant decrease in thyroid hormones T3, T4 with elevated TSH level. Also, MDA level was increased and GPx enzyme activity was decreased. Thyroid glands showed histopathologic, immunohistochemical of Caspase-3 and Ki 67 and morphometric analysis changes. Treatment with both GSE and curcumin alleviated TPTC toxicity, restored thyroid functions and antioxidant profiles to control values more than treatment with only GSE or curcumin as evidenced by the improvement in histopathological, immunohistochemical and morphometric analysis results. **Conclusion:** The toxic effects of TPTC on thyroid gland were reversed partially by supplementation with either GSE or curcumin and reversed nearly to control values with their combined administration. **It is recommended** to do further researches about the combined antioxidants usage during exposure to TPTC.

Key words: Triphenyltin chloride**,** thyroid**,** GSE, curcumin, Caspase-3 and Ki 67**.**

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1. INTRODUCTION

Endocrine disruptive substances are receiving more attention today and are a global concern for many health groups **(Badr El Dine et al., 2017)**. Triphenyltin chloride (TPTC) and Tributyltin (TBT) as an organotin compounds (OTs), are common pollutants that are extensively employed as biocides, agricultural fungicides, and Preservatives for wood. Moreover, they have been used as antimicrobial paints and disinfectants for marine vessels **(Lu et al., 2022)**. They remain in the environment, severely contaminating ecosystems and building up in biological tissues due to the possibility of transmission through marine food chains and intake of tainted seafood **(Badr El Dine et al., 2017).**

As TPTC can directly harm endocrine glands, interfere with the regulation of neurohormones on endocrine function, and alter hormone synthesis and/or bioavailability, it should be considered a serious endocrine disruptor**(He et al., 2021)**.Triphenyltin chloride has been found to be hazardous to male reproduction **(Lu et al., 2022)**, as they are involved in healthy brain development, metabolic management, and many other significant elements of healthy adult physiology. Almost every organ needs thyroid hormones to function properly. So, if the thyroid gland's function is disrupted or thyroid hormone's ability to exert its effects is interfered with, adverse effects may have an impact on development, metabolism, or adult physiology **(Yao et al., 2020).**

According to several studies, both in vivo and in vitro, Mouse cells may experience oxidative damage from the organotin. Oxidative stress, which is brought on by an abundance of reactive oxygen species(ROS), leads to cellular oxidative injury, including lipid peroxidation, protein oxidation, and DNA damage **(He et al., 2021).** Hence, because natural herbs have anti-cancer, anti-oxidant, anti-inflammatory, anti-bacterial qualities, their medical applications are constantly expanding **(Aboul-Fotouh et al., 2018)**.

Also, there was a sharp rise in interest in natural antioxidants in recent years. Natural antioxidants can prevent the ageing process and several chronic diseases like cancer, cardiovascular disease, and cataracts in humans as well as delay the oxidative rancidification of dietary lipids **(Hammoud et al., 2014).** Grape seed extract, is a wholly organic extract created from Vitis vinifera seeds. Grape seed extract has a significant level of flavonoids. The most common of these are proanthocyanidins, which are oligomers of monomeric flavan-3-ol molecules joined by carbon-carbon bonds. The three primary flavan-3-ols present in GSE are (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-O-gallate **(Morsi et al., 2020).** It has been found to have antioxidant properties, with high concentrations of vitamin C, bioflavonoids and vitamin E **(Hammoud et al., 2014).**

Grape seed extract is thought to have a protective impact through controlling cellular oxidative stress, minimizing damage of organ, increasing the antioxidant-oxidant ratio while decreasing the generation of inflammatory mediators. Aside from that, anticancer effects have been reported **(Al-Naely and Shattnan, 2017)**.

One of the most well-known natural polyphenols is curcumin. It is a key component of the turmeric spice, which is made from the rhizome of the herb Curcuma longa Linn. Turmeric is a widely used spice. The anti-inflammatory, antioxidant, anti-cancer, and hypolipidemic effects of curcumin are also present. Moreover, curcumin was reported to be pharmacologically safe for people and animals with very good tolerance, being many times more effective than vitamin E as a free radical scavenger **(Aboul-Fotouh et al., 2018).**

Aim of the Study:

The purpose of this study was to investigate the possible protective effect of GSE and Curcumin either alone or in combination on TPTC induced thyroid toxicity in adult albino rats

II. MATERIALS AND METHODS

II.1 Chemicals:

Triphenyltin Chloride (TPTC): Sigma-Aldrich Chemical Company in St. Louis, Missouri, USA, is where it is purchased. 95% of TPT is pure. Package size 250mg, White to off-white powder, CAS number 639-58-7

Super Pure Grape Seed Extract (GSE) (SYN-00467) 60 capsules per package from Pure Synergy. It contains 150 mg of organic proanthocyanidins per capsule.

Curcumin: A bottle containing 10 gm of yellow curcumin powder could be acquired from Sigma-Aldrich Chemical Company (Catalogue number C1386; Cairo, Egypt).

As a TPTC and curcumin solvent agent, **corn oil** was purchased from commercial sources. **Distillated water** was Solvent for GSE.

II.2 Animals and Experimental design

The Zagazig University's animal house served as the site of this study. There were 54 adult albino rats, each weighing between 150 and 200 g about 6 weeks age. They were kept in typical stainless steel cages under typical environmental settings. They were given regular food and water to drink. Before the trial began, they were housed in appropriate settings for a week to allow for acclimatization. They were handled in accordance with protocols approved with the Zagazig University Animal Use Committee with approval code ZU-IACUC/3/F/348/2022.

Seven groups were created for the animals. All drugs were administered orally to rats via oral gavage daily for 4 weeks

- Group I (Control group): number (N)18 rats
Divided equally and at random into the Ia, Ib, and Ic subgroups:

Group Ia (Negative control): rats received a conventional food and tap water to test the fundamental parameters.

Group Ib (Distilled water control): each rat received distilled water (0.5 ml) (the GSE solvent).

Group Ic (Corn oil control) Each rat received 0.5 cc of corn oil (the solvent for curcumin and TPTC)

- Group II (GSE):N.6 rats
Rats were administered GSE at 150 mg/kg **(Al-Naely and Shattnan, 2021)**, dissolved in 0.5 cc of distilled water

- Group III (Curcumin):N.6 rats
Curcumin was administered orally to rats at dose 100 mg/kg **(Abdelaleem et al., 2018)**, dissolved in 0.5 ml corn oil.

- Group IV (TPTC):N.6rats
TPTC was administered orally to rats at a rate of **(10 mg/kg/day) which represents 1/20 of LD50 OF TPTC in rats according to toxicology data sheet and to (Grote et al., 2004)** dissolved in 0.5 ml corn oil.

- Group V (GSE +TPTC):N.6 rats
GSE was given to rats at dose 150 mg/kg in 0.5 ml of distilled water, along with 10 mg/kg of TPTC in 0.5 ml of corn oil.

- Group VI (Curcumin +TPTC): N.6 rats
Curcumin and TPTC were both given orally to rats at doses of 100 mg/kg/day and 10 mg/kg/day, respectively, dissolved in 1ml corn oil.

- Group VII (GSE + Curcumin + TPTC): N.6rats
Received 150 mg/kg of GSE diluted in 0.5 ml of distilled water. Also, curcumin and TPTC were both given orally to rats at doses of 100 mg/kg/day and 10 mg/kg/day, respectively, dissolved in 1ml corn oil.

The medications were administered at two distinct intervals; GSE and Curcumin were administered in the morning (9 AM) as antioxidants should be given before the toxic intervention to produce the protective effect, while TPTC was administered in the late afternoon (3 PM).

II.3 METHODS:

II.3.1 Body Weight:

Rats in all groups had their body weights measured at the start and at the end of experiment and their weights were recorded.

II.3. 2 Biochemical examination:

Rats were anaesthetized; blood samples were taken, for biochemical analysis.

From each rat's tail vein, blood samples were taken in non-heparinized glass tubes. Centrifugation was used to separate the serum for 15 minutes at 3000 rpm, and then stored at -20 °C

I- **Triiodothyronine (T3),Thyroxine (T4),** and **thyroid-stimulating hormone (TSH)** levels in I-serum were assessed using an ELISA **(Kuriyama et al., 2007).**

II-Oxidative stress parameters

In accordance with **Draper and Hadley's (1990)** approach, serum malondialdehyde (MDA) was quantified using an MDA biodiagnostic kit. Glutathione peroxidase activity was also assessed (GPx) The GPX biodiagnostic kit associated with the procedure described by **(Paglia and Valentine.1967)** was used to measure the GPX activity.

2.3.3Histopathological examination:

The thyroid glands were then removed from the sacrificed rats and processed for histological and immunohistochemical analysis by (**Light microscope).**

A) Hematoxylin and Eosin

Thyroid glands were extracted from rats from different groups, promptly fixed in 10% buffered neutral formalin solution for 24 hours, dehydrated in successively greater ethanol concentrations, washed in xylene, and then embedded in paraffin wax. Using a microtome, 5 m thick paraffin sections were cut (Leica RM 2155, England). Hematoxylin and eosin stains were commonly used to prepare the slices before they were inspected under a microscope **(Suvarna et al., 2018).**

b) Immunohistochemical staining Caspase-3 and Ki 67 of thyroid tissue

Samples of the thyroid measuring 5 micrometres thick underwent immune staining for caspase-3 enzyme (apoptotic cell marker) and the cellular proliferation marker Ki-67.

Sections were kept with anti-caspase 3 (Novus, rabbit polyclonal IgG, Cat. #NB100- 56113) antibodies following incubation with 3% H2O2 solution for 20 min at 27°C. Sections were then exposed to secondary polyclonal IgG antibodies against mice following that (dilution, 1:1,000). Afterwards, the slices were incubated with streptavidin stained with peroxidase from horseradish for 20 min before being washed with PBS **(Kececi et al., 2016).**

And anti-Ki67 [Anti Ki67 antibody: A monoclonal antibody from rabbits (Lab Vision Company labs, USA, RM-9106-R7)] **(Lombardi et al., 2014).**

II.3.4 Morphometric Analysis

In the Anatomy Department of the Zagazig Faculty of Medicine, morphometric analysis was carried out using the image analyzer (an Image J plugin) as follows: Sections of H&E of different studied groups were analyzed morphometrically by measuring diameter of thyroid follicles (mean of 5 reading) in five non -overlapped fields at 100 magnifications.

Positive reaction of Ki-67 and caspase-3 of immune-stained sections was quantitively assessed by measuring area percent of their positivity in five non overlapped fields (X400) in different studied groups.

II.3.5 Statistical Analysis:

The data were reported using the statistical analysis programme GraphPad Prism (version 8 software for Windows) (SD). In that analysis, one-way analysis of variance (ANOVA) was employed to determine statistical significance and group mean using post hoc multiple comparisons. The P value cutoff for significance was 0.05.

III. RESULTS

III.1Mortality and General Observations:

The administered dose of TPTC caused no deaths among the different studied groups of animals during the experiment.

III.2 Body weight (Table-1):

Control group, GSE and curcumin groups showed non-significant difference for body weight.

Regarding the body weight, there was no statistically significant difference among the studied groups before intervention (P value >0.05). After intervention statistically significant difference among the different studied groups was detected (P value < 0.001*). As a result, the weight increased in a statistically very significant way in (TPTC group) compared to all the other groups. There was no discernible difference between GSE + TPTC and curcumin+ TPTC groups. GSE+curcumin +TPTC group revealed that no increase of weight regarding the TPTC group. Also it showed significant decrease in weight compared to GSE +TPTC group and curcumin + TPTC group but still not reached the control level.

III.3 Serological results (Table-2):

Control group, GSE and curcumin groups showed non-significant difference concerning the different biochemical parameters.

Regarding thyroid function (TSH, T3 and T4) levels among the studied groups, between the control groups, there was no statistically significant difference. (P value>0.05). A highly significant increase in TSH level and highly significant decrease in T3 and T4 levels were recorded in TPTC group compared to all the other groups. Treated groups GSE+TPTC and curcumin+ TPTC showed significant improvement in TSH, T3 and T4 levels but GSE+ curcumin +TPTC showed the best improvement , however still not reached the control level. When compared to all other groups, the MDA level in the (TPTC group) increased by a highly significant amount. Concerningly, the (TPTC group) experienced a significant drop in GPX activity as compared to all the other groups.

Combined group showed significant improvement in MDA level, GPX activity compared to both GSE+TPTC group and curcumin +TPTC group but still not reached the control level.

Table (1): Statistical analysis of body weight measurement of different groups at the start and at the end of the experimental procedures by ANOVA and post hoc multiple comparisons tests.

Parameters / Groups	Weight before (gm)	Weight after (gm)
Group I (Control) mean±SD	146.66±3.5	151.66±5.3
Group II (GSE) mean±SD	149.38±5.19 Ns[a,c]	153.16±6.36 Ns[a,c]
Group III (Curcumin) mean±SD	148.66±3.83 Ns[a,b]	148.66±3.83 Ns[a,b]
F value	0.857	0.258
Group IV (TPTC) mean±SD	147.5±3.27 Ns[a,b,c]	235.5±10.72 P<0.001[a,b,c]
Group V (GSE+ TPTC) mean±SD	148.5±3.61 Ns[a,b,c,d]	200.0±23.2 P<0.001[a,b,c,d]
Group VI (Curcumin + TPTC) mean±SD	145.16±2.78 Ns[a,b,c,d,e]	192.33±10.67 P<0.001[a,b,c,d] Ns[e]
Group VII(GSE+curcumin +TPTC) mean±SD	147.83±2.40 Ns[a,b,c,d,e,f]	170.5±18.0 P<0.001[b,c,d] Ns[a e,f]
F value	0.995	26.89

P < 0.05 = significant

a = significant versus group I, b = significant versus group II, c= significant versus group III, d= significant versus group IV, e= significant versus group V, f= significant versus group VI.

Table (2): Comparing thyroid function tests, MDA and GPX among the studied groups by ANOVA and post hoc multiple comparisons tests.

Groups	T3 (µg/ml)	T4 (ng/ml)	TSH (IU/ml	MDA nmol/ml	GPx (ng/ml)
Group I (Control) mean±SD	0.93±0.07	4.75±0.5	0.44±0.03	10.8±1.96	163.88±0.07
Group II (GSE) mean±SD	1.06±0.12 $Ns^{a,c}$	4.5±0.23 $Ns^{a,c}$	0.51±0.06 $Ns^{a,c}$	11.75±0.75 $Ns^{a,c}$	166.66±56.71 $Ns^{a,c}$
Group III (Curcumin) mean±SD	1.01±0.16 $Ns^{a,b}$	4.85±0.24 $Ns^{a,b}$	0.50±0.06 $Ns^{a.b}$	9.83±1.47 $Ns^{a,b}$	167.66±4.58 $Ns^{a,b}$
F value	0.899	1.59	2.58	2.96	0.715
Group IV (TPTC) mean±SD	0.24±0.02 $P<0.001^{a,b,c}$	1.53±0.25 $P<0.001^{a,b,c}$	5.33±0.54 $Ns^{a,b,c}$	53.33±10.8 $P<0.001^{a,b,c}$	94.68±13.68 $P<0.001^{a,b,c}$
Group V (GSE+ TPTC) mean±SD	0.49±0.07 $P<0.001^{a,b,c,d}$	2.86±0.36 $P<0.001^{a,b,c,d}$	2.4±0.74 $Ns^{a,b,c,d}$	23.33±1.86 $P<0.001^{a,b,c,d}$	133.33±6.28 $P<0.001^{a,b,c,d}$ Ns^{e}
Group VI (Curcumin + TPTC) mean±SD	0.47±0.03 $P<0.001^{a,b,c,d}$ Ns^{e}	2.9±0.18 $P<0.001^{a,b,c,d}$ Ns^{e}	2.01±0.61 $Ns^{a,b,c,d,e}$	22±1.41 $P<0.001^{a,b,c,d}$ Ns^{e}	133.33±6.28 $P<0.001^{a,b,c,d}$ Ns^{e}
Group VII(GSE+curcumin +TPTC) mean±SD	0.84±0.03 $P<0.001^{b,c,d}$ $Ns^{a,e,f}$	3.63±0.33 $P<0.001^{b,c,d}$ $Ns^{a,e,f}$	1.04±0.11 $Ns^{a,b,c,,d,e,f}$	16.2±1.77 $P<0.001^{b,c,d}$ $Ns^{a,e,f}$	153.16±5.84 $P<0.001^{b,c,d}$ $Ns^{a,e,f}$
F value	170.5	53.1	85.7	64.7	67.1

Ns = non-significant (P > 0.05), P < 0.001=statistically significant, a = versus group I, b = versus group II, c=versus group III, d=versus group IV, e=versus group V, f=versus group VI

TSH: thyroid-stimulating hormone T3: Triiodothyronine T4: Thyroxine MDA: serum malondialdehyde .GPx: Glutathione peroxidase.

III.4 Histopathological results

Light microscope (H&E staining):

Upon evaluation of the control groups' thyroid regions, GSE group as well as curcumin group, they revealed normal architecture. Thyroid glands appeared formed of different sized follicles lined with low cuboidal epithelium with rounded nucleus. The follicular lumen is filled with an acidophilic colloid. Inter follicular cells existed in between the follicles with few thin walled blood vessels **(Figure.1a)**. On the other hand, thyroid section of the TPT group showed different sized follicles, some appear atrophied and others with empty lumen. Lining of the follicles appeared vacuolated and lumen of few follicles had scanty acidophilic colloid. Inter follicular cells appeared vacuolated with congested blood vessels in between **(Figure.1b).** On administration of GSE as well as curcumin, thyroid sections showed apparent improvement. Several sized follicles of various sizes were seen in thyroid sections; the majority of them were bordered with a low cuboidal lining and filled with acidophilic colloid, few appear atrophied while others appeared with empty lumen and vacuolated lining .some inter follicular cells were vacuolated with congested blood vessels in between**(Figure.1c&d).** Thyroid sections of the (GSE + curcumin +TPTC) group showed obvious improvement, as most of follicles were filled with acidophilic colloid and lined with low cuboidal lining and few with vacuolated lining with detached lining inside the lumen. Few inter follicular cells appeared vacuolated with few congested blood vessels in between **(Figure.1e).**

Immunostaining for Caspase-3

In the cytoplasm of the follicular lining, control, GSE and curcumin groups barely exhibited no positive immunoreaction for caspase-3 expression (Figure.2a). Caspase-3 immunoreaction was strongly positive in the cytoplasm of follicular lining cells and less so in interfollicular cells in TPTC-exposed animals (Figure.2b). This reaction became significant in the GSE+ TPTC and curcumin +TPTC groups (Figure.2c, d), and there was a mild positive immunoreaction for caspase-3 in the GSE + curcumin +TPTC group (Figure2e.).

Immunostaining for Ki67.

There was minimal positive immunoreaction for ki 67expression in the nuclei of follicular lining of the groups of control, GSE, and curcumin (Figure.3a). Meanwhile, the nuclei of follicular cells from the TPTC group displayed a high positive immunoreaction for Ki67 (Figure.3b). (GSE+ TPTC and curcumin +TPTC groups) showed moderate positive immunoreaction for Ki67 (**Figure.3c&d)** and weak immunoreactive response for ki 67 in GSE+ curcumin +TPTC group (Figure3e.).

III.4Morphometric analysis

- **Follicular diameter assessment**
  Analysis of the follicular diameter in different groups revealed non - significant difference between control and both GSE and curcumin groups, While TPTC group showed significant decrease of follicular diameter than all other groups. Follicular diameter increased significantly in GSE+TPTC, curcumin+ TPTC and GSE+ curcumin +TPTC groups than TPTC group but less than the control group (Table.3).
- **Area % of caspase3 and Ki67 immune expression**
  Analysis of area % of caspase3 and Ki67 immune expression in different groups revealed non -significant difference between control and both GSE and curcumin groups. While, in TPTC group showed significant increase of area percentage expression of caspase3 and Ki67 than the all other groups. This percentage substantially dropped in GSE+TPTC, curcumin+ TPTC and GSE+ curcumin +TPTC groups than TPTC group but still more than control group (Table.3).

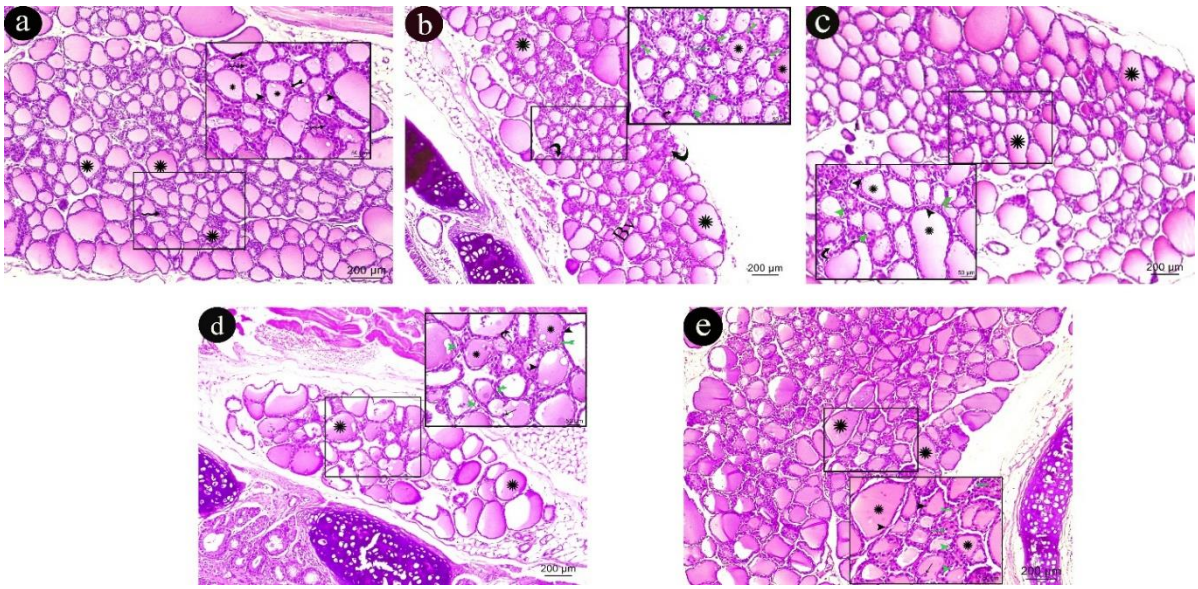

Figure 1 [a-e]: Representative photomicrographs of H&E-stained sections of thyroid of different experimental groups. **[A]control group** shows different sized follicles lined with low cuboidal epithelium with rounded nucleus (**arrow head**). The follicular lumen is filled with an acidophilic colloid (*). Inter follicular cells existed in between the follicles with few thin walled blood vessels (**tailed arrow**). **[b] TPTC group** shows some atrophied follicles **(curved arrow),** and others with empty lumen **(green curved arrow)** with vacuolated Lining **(green arrow head)** and scanty acidophilic colloid **(green curved arrow)**. Inter follicular cells appeared vacuolated **(green zigzag arrow**) with congested blood vessels (**green tailed arrow**) in between. **[c] GSE+ TPTC** and **[d] curcumin+ TPTC** show different sized follicles are lined with a low cuboidal lining(**arrow head**) and filled with acidophilic colloid (*), few appear atrophied **(curved arrow**) while others appeared with empty lumen**(green curved arrow)** and vacuolated lining**(green arrow head)** with congested blood vessels in between follicles (**green tailed arrow**) and detached cells inside follicles(arrow). **[e] GSE+ curcumin+ TPTC group.** show different sized follicles are lined with a low cuboidal lining(**arrow head**) and filled with acidophilic colloid (*), few with vacuolated lining**(green arrow head)**, congested blood vessels in between follicles (**green tailed arrow**) and detached cells inside follicles**(arrow (H&E x 100 & inset magnification x400).**

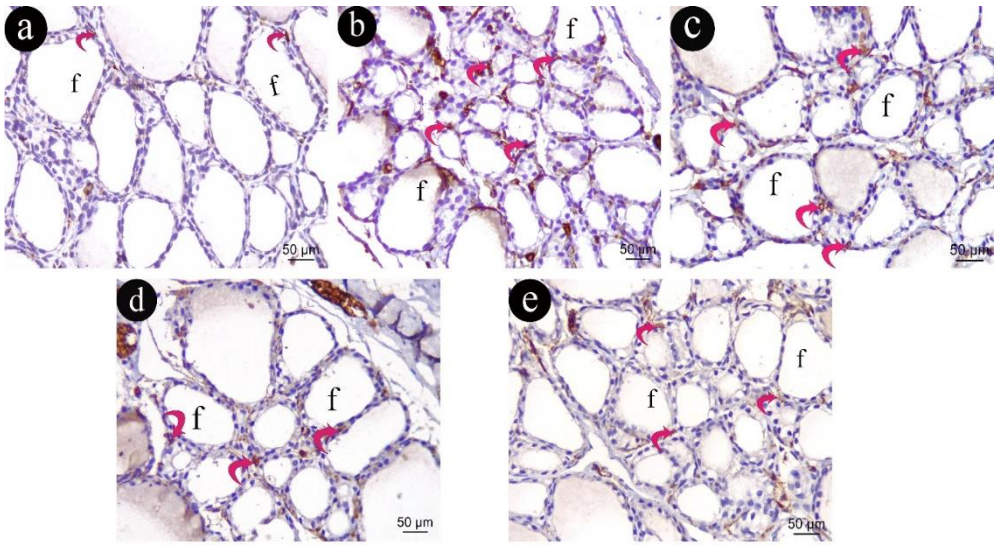

Figure 2 [a-e]: Representative photomicrographs of **Caspase-3 immune-stained** sections of thyroid of different experimental groups. **[a] control group**, **[b] TPTC group**, **[c] GSE+TPTC group** , **[d] curcumin+ TPTC group**, and**[e] GSE+ curcumin+ TPTC group**. Thyroid follicles are **(f)** and positive reaction (**curved red arrow**) (**Immunoperoxidase technique x 400).**

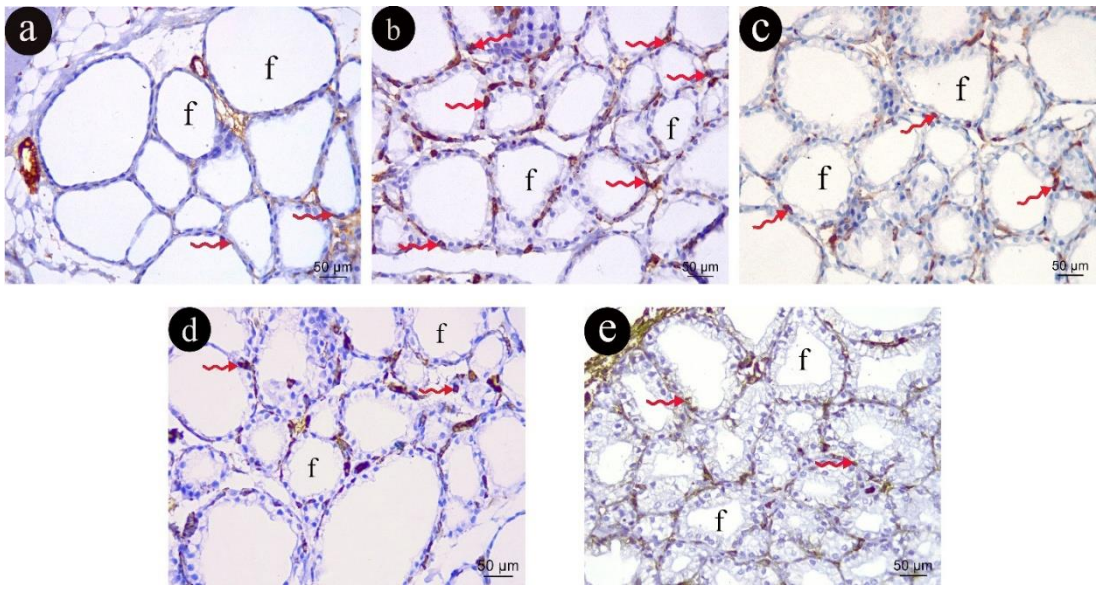

Figure 3 [a-e]: Representative photomicrographs of **Ki-67** -stained sections of thyroid of different experimental groups. **[a] control group**, **[b] TPTC group**, **[c] GSE +TPTC group** , **[d]** thyroid section of the **curcumin+ TPTC group**, and**[e] GSE+ curcumin+ TPTC group**. Thyroid follicles are **(f)** and positive reaction (**zigzag red arrow) (Immunoperoxidase technique x 400).**

Table (3): Comparing follicular diameter (µm), Caspase3 area % and ki67 area % among the studied groups by ANOVA and post hoc multiple comparisons tests.

Groups	Follicular diameter (µm)	Caspase 3 area%	Ki 67 area %
Group I (Control) mean±SD	51.10±3.488	2.988±0.5362	1.783 ±0.3098
Group II (GSE) mean±SD	51.23±3.447 Ns[a]	2.951±0.5853 Ns[a]	1.833 ± 0.2867 Ns[a c]
Group III (Curcumin) mean±SD	51.27±3.445 Ns[a,b]	2.955±0.5288 Ns[a,b]	1.818 ±0.2842 Ns[a,b]
Group IV TPTC mean±SD	30.11±1.375 P<0.001[a,b,c]	11.98±1.173 P<0.001[a,b,c]	10.38 ±0.7611 P<0.001[a,b,c]
Group V (GSE+ TPTC) mean±SD	36.44±1.882 P<0.001[a,b,c,d]	7.448±0.705 P<0.001[a,b,c,d]	6.078±0.436 P<0.001[a,b,c,d]
Group VI (Curcumin + TPTC) mean±SD	38.37±1.589 P<0.001[a,b,c,d] Ns[e]	7.275±0.8719 P<0.001[a,b,c,d] Ns[e]	5.975 ± 0.4766 P<0.001[a,b,c,d] Ns[e]
Group VII(GSE+curcumin +TPTC) mean±SD	42.80±2.092 P<0.001[a,b,c,d,e,f]	4.621±0.7279 P<0.001[a,b,c,d,e,f]	4.021 ± 0.6816 P<0.001[b,c,d,e,f]

Ns = non-significant (P > 0.05), P < 0.001=statistically significant, a = versus group I,
b = versus group II, c=versus group III, d=versus group IV, e=versus group V, f=versus group VI

IV. DISCUSSION

Hormonal problems have dramatically increased over the past few decades, and it has been proposed that part of the cause of this burden on communities is raising exposure to endocrine disrupting chemicals (EDCs). There are, however, limited reports on how TPTC affects thyroid tissues. So, this piqued our curiosity to carry out an oral toxicity study to learn more about TPTC impact on follicular cells of the thyroid. The thyroid gland seems to be particularly vulnerable to ED side effects. Certain environmental toxins have the power to interfere with the production, metabolism, and biological consequences of THs as well as other aspects of the HPT axis. Given that every organ's shape roughly corresponds to how well it functions, Compared to serum T3 and T4 levels, thyroid gland histology is a more reliable early predictor of glandular activity. **(Badr El Dine et al., 2017**)The current study revealed that the body weight of the TPTC-treated rats rose in comparison to the control group. Because it triggers adipogenesis by signalling between the retinoid X receptor (RXR) and peroxisome proliferator-activator receptor gamma(PPAR), TPTC has been labelled as an obesogen, **(Pivonello et al., 2020)**.According to experts, hormonal alterations brought on by faulty hypothalamic-pituitary function can also account for the rise in body weight **(Marques et al., 2018)**. It is crucial to understand that there is a connection between obesity and thyroid health. Changes in body weight and composition are linked to thyroid dysfunction. Often, overweight patients with hypothyroidism present **(Sanyal and Raychaundhuri, 2016).** Obesity is usually linked to comorbidities like hypertension, dyslipidemia, and coronary heart disease and is seen as a global epidemic with multiple causes **(WHO, 2009)**. Since rises in the quantities of

some chemical chemicals in the environment have accompanied increases in the frequency of obesity in recent decades, the rising number of fat individuals worldwide may not be simply attributable to a sedentary lifestyle and hypercaloric meal intake **(Santos-Silva., 2018).**As a result, it is conceivable that endocrine disruptors have an impact on adipose tissue, causing obesity and becoming overweight. Studies that categorise endocrine disruptors as obesogens in general **(Grün and Blumberg, 2009; Schneider et al., 2014)** have supported this theory. The designation of TPTC as an obesogenic endocrine disruptor is therefore likely **(Chamorro-Garca et al., 2013; Grün, 2014).** Exposure to these substances is widespread and regular, and even very small doses of EDs have the potential to have negative health effects on the endocrine system **(Gao et al., 2015)**. So, a deeper comprehension of TPTC's effects on the HPT axis is essential to comprehending its endocrine and metabolic toxicity. The triphenyltin chloride-treated group in the current study displayed a highly significant decrease in blood T3, T4, as well as a highly significant increase in serum TSH when compared to the control group. This substantial decrease in these hormone levels suggests that TPTC inhibits thyroid function. There are several theories to explain thyroid toxicity brought on by TPTC. Previous researchers **(Santos-Silva, 2018)** documented a similar alteration in the hormones levels and related it to the antithyroid impact of TPTC. This was further corroborated by a histological study of follicular cells, which showed evident structural abnormalities indicative of increased activity in thyroid follicles of this group as a result of excessive TSH secretion to make up for the dropping levels of T3 and T4.Swollen vacuolated follicular cells, sparse colloid, and degenerating follicles were all signs of these alterations. Increased Ki 67 immune expression was also associated with congested blood arteries. Further supporting these alterations were morphometric and statistical analyses that revealed a significant decrease in the mean thyroid follicle diameter in comparison to the respective controls. According to their findings, **(Santos Silva et al., 2018)** stated that EDs are powerful disruptors of thyroid function because they change how the hypothalamus-pituitary-thyroid axis is regulated and functions. Also, the current investigation showed small-sized, empty follicles along with disruption of the gland's typical architecture. Apoptosis is also indicated by elevated Caspase 3 immune expression, which is consistent with **(Zhang et al., 2012)**'s view that darkly stained nuclei appearance is a symptom of apoptosis. When researching the impact of TPTC on the thyroid gland of zebra fish, **(Hori et al., 2021)** observed similar results. They claimed that TPTC could severely harm thyroid tissues. The follicular region shrank, colloid levels dropped, and the follicles became malformed as a result of this damage. Furthermore, as ROS can adversely affect crucial elements of thyroid glandular activity, the increased oxidative stress brought on by a compromised antioxidant defence status may be to blame for the cascade of responses that leads to TPTC-induced reproductive toxicity. A highly significant drop in serum GPx activity and a highly significant increase in serum MDA level were seen in the current study when the TPTC-treated group was compared to the control group. These results are consistent with earlier research by **(Zhang et al., 2021)** that hypothesised that one of the key processes underlying TPTC toxicity that damages mitochondria and other structures involves the production of ROS. **(Li et al., 2018)** showed that in vitro exposure to TPTC enhanced ROS production and caused cell death in rats. They reported that TPTC produced an increase in lipid peroxidation, a decrease in GPx, and a loss of antioxidant defence enzyme activity. According to **Lu et al. (2022)** TPTC is linked to oxidative stress, a lack of antioxidant defence enzymes, and an excess of MDA. GSE and curcumin treatment together restored normal body weight. Also, when compared to the TPTC group, the GSE and curcumin treated groups had significantly higher mean values of T3 and T4

and much lower mean values of TSH. These findings suggested that using GSE with curcumin might improve thyroid function more effectively. In addition, there was a clear histological advancement over the TPTC group. The majority of the follicles had a single layer of what appeared to be normal follicular cells lining them, and their diameters had significantly increased while the expression of the immune cells caspae3 and Ki67 had significantly decreased. A highly substantial drop in blood MDA and an increase in serum GPx activity were observed. In the GSE+TPTC and curcumin+TPTC groups when compared to the TPTC group, demonstrating a profound antioxidant role for GSE and curcumin. Co-administration of GSE and curcumin with TPTC also significantly attenuated the effect of TPTC on biochemical markers of oxidative stress and lipid peroxidation Curcumin and GSE both have antioxidant properties, as shown in numerous researches previously. According to **(Morsi et al .,2020),** GSE's possible actions against oxidative stress and the reduction of free radical production, which preserves the compromised antioxidant defence system, may both contribute to its protective impact. Similar outcomes were also mentioned by **(El-Beshbishy et al., 2009). Al-Naely et al., 2017** reported that grape seed extract played an important protective role in preventing liver and kidney damage brought on by thyroid disorders and their medications carbimazole and L-thyroxin. They recommended using grape seed extract for patients with subclinical thyroid disorders as well as using it in conjunction with thyroid therapy for patients with thyroid disorders**. El-Ashmawy and Bayad (2016)** attributed the protective properties of grape seed to the phenolic and flavonoid compounds and their capacity to treat inflammation, activate cellular defences against oxidative stress, and maintain mitochondria. These properties limited accelerated programmed death and increased glutathione levels within the cell. According to studies, grape seed extract is a potent free radical blocker and simultaneously activates the antioxidants in the enzymes **(Ali et al., 2015).** According to research by **(Hammoud et al., 2014),** the GSE proanthocyanidins' notable capacity to scavenge free radicals may be the mechanism underlying the organ protection provided by GSE. They observed that GSE could minimise organ damage due to its ability to balance the oxidant-antioxidant status and regulate the release of inflammatory mediators **(Sehirli et al., 2008).** It has been shown that GSE has conjugated structures between the 3-OH free groups of polymeric skeletons and the B-ring catechol groups, making them effective metal chelators and free radical scavengers **(Yilmaz and Toledo, 2004).**

In order to prevent DNA damage, it has been shown that the aroxyl radical generated by GSE as it scavenges free radicals is more stable than that generated by other polyphenolics. **Devi et al. (2006)** found that proanthocyanidin, a naturally occurring antioxidant from GSE, was effective in up-regulating the antioxidant defence system by attenuating lipid peroxidation. According to **Aboul-Fotouh et al. (2018),** curcumin is a bifunctional antioxidant that has both direct and indirect effects by promoting the production of antioxidant enzymes and scavenging ROS. The capacity of curcumin to interact with a variety of molecular targets implicated in inflammation has been related to its anti-inflammatory activities. By reducing the activity of certain kinases, it modifies the inflammatory response. In a prior study**, Sourour et al. (2014)** found that turmeric extract boosted cells' antioxidant defence and reduced ROS' harmful effects. In conclusion, our results clearly showed that treatment with both GSE and curcumin alleviated the toxic effect of TPTC and restored both antioxidant profile and thyroid functions to normal more than treatment with only GSE or only curcumin as evidenced by the improvement in oxidative stress parameters and thyroid function parameters and histology

V. CONCLUSION:

The toxic effects of TPTC on thyroid gland were reversed partially by supplementation with

either GSE or curcumin and reversed nearly to control values with their combined administration .

VI. RECOMMENDATIONS:

- Grape seed extract and curcumin can be used for protection against TPTC -induced thyroid toxicity.
- Further studies are needed to:
- Identify other mechanisms of TPTC -induced thyroid toxicity.
-Clarify additional information regarding impact of GSE-TPTC and curcumin- TPTC combinations with longer duration, larger sample size and larger protective doses than the used in our study.

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التأثير الوقائى المحتمل لمستخلص بذور العنب و الكركمين على سمية الغده الدرقيه التى يسببها التريفينيلتين كلوريد فى الجرذان البيضاء البالغه

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الملخص العربى

مقدمه: تعد ماده التريفينيلتين كلوريد من مركبات القصدير العضوي حيث توجد فى منتجات كثيره من أشهرها المنظفات والمبيدات الحشريه وايضا تدخل فى صناعه الجلود .وتعتبر مركبات القصدير واحده من المسببات فى تدمير الغدد الصماء.تتعارض ماده التريفينيلتين كلوريد مع السيطرة العصبيه الهرمونية لوظائف الغدد الصماء .تتعرض الثدييات لتأثير هذه المركبات من خلال تناول الأطعمه البحريه.

الهدف :ويعد الهدف من الدراسه هو معرفه التأثير الوقائى لكل من مستخلص بذور العنب والكركمين على سمية الغده الدرقيه المحدثة نتيجة التعرض لمركب التريفينيلتين كلوريد فى الجرزان البيضاء البالغه.

المواد والطرق المستخدمة:أجريت الدراسه على 54 من الجرزان البيضاء البالغه .المجموعه الأولى هى المجموعة الضابطة وتحتوى على 18من الجرزان وتنقسم إلى 3مجموعات ،مجموعه لا تتلقى أى علاج ومجموعه تتلقى 5. مل مياه مقطرة ،ومجموعه تتلقى 5.مل من زيت الذرة يوميا لمده 4 أسابيع وتستخدم هذه المجموعه لقياس الوظائف الحيويه للجرزان حيث أنها لا تتلقي اى علاج .وباقى العدد 36 من الجرزان قد تم تقسيمها بشكل متساوى على 6 مجموعات كل مجموعه تحتوى على 6 من الجرزان وتتلقى العلاج يوميا عن طريق الفم لمده 4 اسابيع.المجموعه الثانيه تتلقى فيها الجرزان لمستخلص بذور العنب بجرعه 150 مجم/كجم يوميا .المجموعه الثالثه تأخذ فيها الجرزان للكركمين بجرعة 100مجم/كجم/يوميا .أما المجموعه الرابعه فتتلقى الجرزان فيها ماده التريفينيلتين كلوريد بجرعة 10مجم/كجم /يوميا والمجموعه الخامسه تتعرض الجرزان مع مستخلص بذور العنب وماده التريفينيلتين كلوريد.والمجموعه السادسه تتعرض للكركمين و ماده التريفينيلتين كلوريد.واخيرا المجموعه السابعه تتلقي كلا من مستخلص بذور العنب والكركمين مع ماده التريفينيلتين كلوريد.بنهايه مده الأربعه أسابيع تم تسجيل وزن الجرزان وسحب مصل الدم لقياس هرمونات الغده الدرقيه ودلالات الأكسده (المالون ديالدهيد والجلوتاثيون بيروكسيديز) وتم ذبح الجرزان وتحضير أنسجه الغده الدرقيه للفحص النسيجى بصبغه الهيماتوكسيلين والإيوسين . وتم إجراء الفحص المناعى لبروتين كاسبيس3 وال67 Ki بواسطه المجهر الضوئى. وتم عمل دراسه مورفومترية.

النتائج:وقد أظهرت النتائج أن ماده التريفينيلتين كلوريد تؤدى إلى نقص فى معدلات هرمونات الغده الدرقيه T3,T4 وإرتفاع فى معدل هرمون TSH ،وايضا زياده دلالات الأكسده بالدم . وقد أدت إلى تغيرات نسيجية وكيميائيه مناعيه وتغيرات فى دراسه المورفومترية فى أنسجه الغده الدرقيه .وأيضا أظهرت النتائج أن المجموعه التى تلقت مستخلص بذور العنب والكركم سويا أدت إلى تحسن وظائف الغدة الدرقية وتحسن نسبه دلالات الأكسدة.وتعد هذه النتائج أفضل من نتائج المجموعات التى تلقت كلا من مستخلص بذور العنب والكركمين على حده وقد ثبت هذا عن طريق التحسن فى الدراسه النسيجية والكيميائيه المناعيه والدراسه المورفومترية

الإستنتاج:ومن هذا نستنتج أن التأثير السام لماده التريفينيلتين كلوريد على الغده الدرقيه يتحسن جزئيا بإستخدام مستخلص بذور العنب والكركمين على حدة.ولكن يتم التحسن لمستويات المجموعة الضابطة بإستخدام كلا من مستخلص بذورالعنب مع الكركمين سويا.

التوصيات:ينصح بإجراء مزيد من الأبحاث حول الاستخدام المشترك لمضادات الأكسدة أثناء التعرض لماده التريفينيلتين كلوريد