

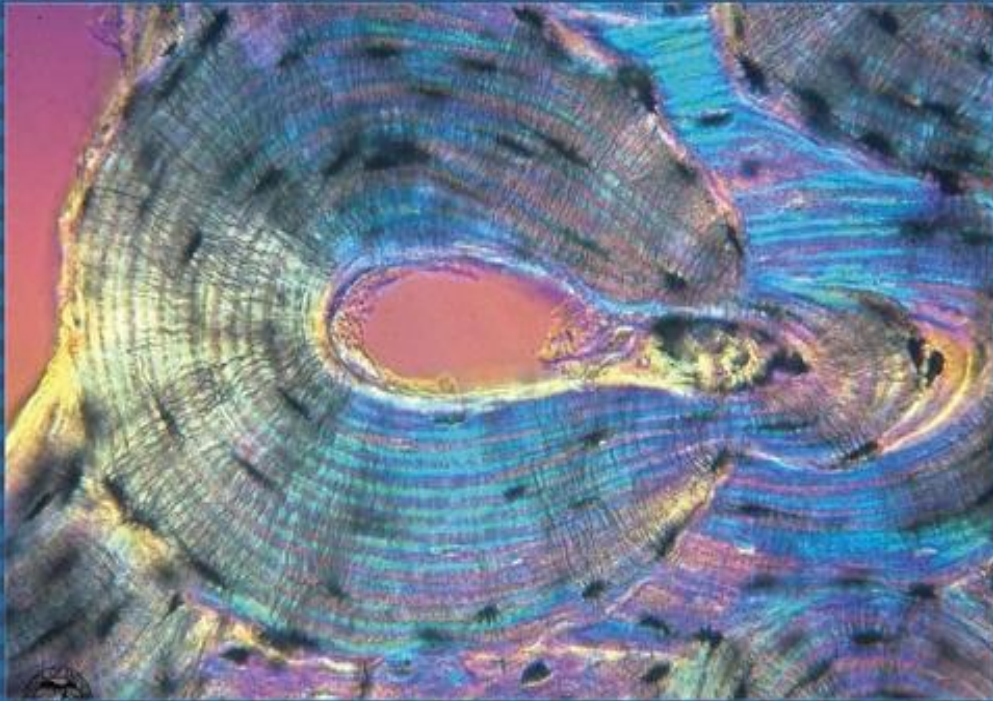


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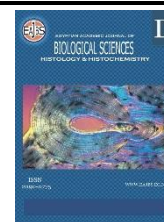


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## Modulatory Efficiency of *Echinacea purpurea* Extract on Hyperthyroidism Modeled Rats

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### ABSTRACT

**Background and objective:**Thyroid hormones are crucial for growth, metabolism, thermoregulation, and development. Hyperthyroidism is a thyroid disorder characterized by over secretion of thyroid hormones that lead to raise in the metabolic rate and oxygen utilization in several tissues. *Echinacea purpurea* (EchEE) is a significant medicinal plant with several pharmacological benefits. **Aim of the work:** This study was conducted to evaluate the modulatory efficiency of *Echinacea purpurea* ethanolic extract on hyperthyroidism-modeled rats. **Materials and methods:** Both normal and hyperthyroidism animals were classified into four groups (10 rats each) as follows: 1) normal animals without any treatments and served as normal control; 2) normal animals orally administrated with EchEE (465 mg/kg/day) dissolved in water for 30 days; 3) untreated hyperthyroidism-modeled animals; 4) hyperthyroidism-modeled animals treated with EchEE for a similar period. **Results:** Treatment of the hyperthyroidism-modeled animals with EchEE, resulted in a significant reduction in the serum level of total T3, total T4, free T3 and free T4 hormones coupled with a remarkable elevation in TSH level. EchEE succeeded also in ameliorating the histological and immunohistochemical picture of the thyroid gland. Also, it improved liver and kidney functions and decreased blood sugar levels. **Conclusions:** This study concluded that EchEE efficiently improved the pathophysiological architecture of the hyperthyroid gland, this was achieved from the marked decline in its hormone with a notable raise in TSH. This effect could be mechanized through the antioxidant characteristics of EchEE constituents. This extract could be a promising medical agent for human health; additional research is needed.

### INTRODUCTION

Thyroid hormones are crucial for growth, metabolism, thermoregulation, and development (Lazar, 1993; Mo Kim *et al.*, 2012). Thyroid and pituitary gland tumors, however, cause thyroid cells to generate extra hormones under several pathological situations, such as Graves' disease, which leads to a hyperthyroid state (Gulcelik *et al.*, 2006; Kim *et al.*, 2012). Changes in these hormone levels cause a variety of health issues in addition to changed basal metabolic rate, more specifically, hyperthyroidism (Kim *et al.*, 2012).

The endocrine condition known as hyperthyroidism is defined by increased thyroid hormone (T3 and T4) release, which causes a rise in basal metabolic rate and oxygen consumption in many tissues (Mannaa *et al.*, 2021).

The hypermetabolic state resulting from hyperthyroidism is associated with various degrees of oxidative stress in the organism. Oxidative damage to lipids, proteins, and DNA, indicative of oxidative stress, was found in hyperthyroid rats (Duntas, 2002; Venditti and Meo, 2006).

In the pharmaceutical business, medicinal plants are known to offer a variety of biological and pharmacological properties that are extremely valuable for the discovery and development of medications with notable efficacy and tolerable side effects. *Echinacea purpurea*, a member of the Asteraceae family, is a significant medicinal plant with several pharmacological benefits (Lee *et al.*, 2009; Khalaf *et al.*, 2019). The active components of *Echinacea purpurea* include carbohydrates, glycosides, alkaloids, alkyl amides, and polyacetylene (Lalone *et al.*, 2007; EL-Sherbiny *et al.*, 2021), sitosterol, cynarine, caffeic acid, chlorogenic acid, echinacoside, and cichoric acid. They are all potent antioxidants that effectively scavenge free radicals and reduce the production of nitric oxide free radicals (Zhao *et al.*, 2010; Motamedi *et al.*, 2018). The choice of anti-hyperthyroid drugs is not based on their relative treatment efficacy, but instead on consideration of side effects and risk of profound clinical consequences, in particular teratogenicity and serious liver injury.

Therefore, the main goal of the present study was to explore the possible modulatory potential of *Echinacea purpurea* extract on hyperthyroid-modeled adult rats.

## MATERIALS AND METHODS

### Plant and Ethanolic Extraction:

Moench (*Echinacea purpurea* L; family: Asteraceae) flowers were obtained from a local supplier in Cairo, Egypt; The plant was identified and was found carrying taxonomy serial number 3728. The dry flowers were grinded and soaked in ethanol (70%) at ratio of (1:5 w/v) for seven days. Then, the mixture was filtered using Whatman 1 filter paper. The solvent was evaporated using a rotatory evaporator, while the moisture residuals were removed through lyophilization process with freeze drier (Todd *et al.*, 2015). Dry *Echinacea purpurea* ethanolic extract (EchEE) was calculated for its yield (g extract /100g crude powdered flowers), however, the obtained dry EchEE was stored at 4 -20 °C until *in vitro* assessments and further use.

### EchEE Radical Scavenging Activity Using DPPH Assay:

Radical scavenging activity (RSA) of EchEE was estimated as previously described (Nogala- Kalucka *et al.*, 2005). Certain amount of EchEE was dissolved in methanol to obtain a concentration of 200 ppm. Then a 200µL of this solution was completed to 4 ml methanol, then 1 ml DPPH methanolic solution ( $6.09 \times 10^{-5}$  mol/L) was added; 10 min later, the absorbance of this mixture was recorded at 516 nm against the absorbance of the blank (1 ml of DPPH solution plus 4 ml methanol) was measured. Both test and blank samples were measured in triplicates. RSA was calculated according to the equation:

$$(\text{RSA}\% = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100).$$



**EchEE Total Phenolics Content:**

Total phenolic content of EchEE was evaluated according to Jayaprakasha *et al.* (2003). In brief, 5 mg of the extract was dissolved in a 10 ml mixture of acetone and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold diluted Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate solution (7.5%). After 30 minutes at room temperature, the absorbance was measured at 765 nm using V-530 UV/visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents was carried out using standard curve of catechin.

**EchEE Reducing Power:**

Ferric-reducing power of EchEE was determined according to the method described by Sethiya *et al.* (2014). The reducing power of the extract was calculated as equivalent to ascorbic acid from the standard curve of ascorbic acid.

**Experimental Design:**

Adult male albino rats (180-250g) were obtained from the animal colony, National Research Centre, and placed in suitable polyethylene plastic cages with free access to food and water one week before the experiment began. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to standard guidelines of the ethical committee of National Research Centre. Hyperthyroidism rats were modeled as described by Carageorgiou *et al.* (2007) by daily subcutaneous injection of L-thyroxine (Eltroxin™ 100mg, GlaxoSmithKline Co, Bomorange Building, Cairo, Egypt, dissolved in saline) at dose of 250 microg/kg/day for 14 consecutive days. After induction of hyperthyroidism, both normal and modeled animals were classified into the four groups (10 rats each) as follows: 1) normal animals without any treatments and acting as

normal control; 2) normal animals orally administrated with EchEE (465 mg/kg/day) dissolved in water (Mao *et al.*, 2021) for 30 days; 3) untreated hyperthyroidism modeled animals; 4) hyperthyroidism modeled animals treated with EchEE for a similar period.

**Blood and Tissue Sampling:**

All animals were weighed at the end of the experiment, then fasted overnight, and then blood samples were collected from the retroorbital venous plexus following inhalation of diethyl ether anesthesia, and divided into 2 portions per each sample: heparinized portions for the determination of hemoglobin, and the other for clear serum which was separated, divided into aliquots, and stored at -80°C until biochemical determinations.

After blood collection, each animal was sacrificed by sudden decapitation, then a part of the liver was dissected out each animal, washed with saline, dried on filter paper, and homogenized in 50 mmol/L phosphate buffer (ice-cold) solution (pH 7.4) to give 10% w/v homogenate. The homogenate was coolly centrifuged at 3000 rpm, and the clear supernatant was separated, divided into aliquots, and stored at -80 °C for further determination of the oxidative stress markers. Immediately, the thyroid gland was dissected out and kept in buffered formalin-saline solution (10%) for the histopathological and immunohistochemical examination which was carried out by cutting 5µm thick paraffin sections and then stain with hematoxylin and eosin (Drury and Wallington, 1980) and examined with a light microscope.

**Biochemical Determination:**

Serum thyroid stimulating hormone (TSH), total triiodothyronin (T3) and total thyroxine (T4) levels were measured by enzyme-linked immunosorbent assay (ELISA) using rat ELISA kits purchased from MyBiosource Co, San Diego, USA.

Serum-free triiodothyronine (FT3) and free thyroxine (FT4) levels were estimated by ELISA technique using rat ELISA Kits purchased from Alpha Diagnostic International, Texas78244, USA. Serum interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13) and tumor necrosis factor-alpha (TNF- $\alpha$ ) levels were determined by ELISA technique using kits manufactured by Assaypro Co., USA. Serum glucose and blood hemoglobin (Hb) concentrations were determined colorimetrically using reagent kits obtained from Biodiagnostic Co. (Egypt).

Serum liver and kidney functions were determined spectrophotometrically to evaluate the safety of EchEE. The concentrations of creatinine and serum urea were assessed using reagent kits obtained from Biodiagnostic Co. (Egypt). The activity of the serum enzymes aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were measured using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostica mbH, Germany. Hepatic glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities, as well as the levels of glutathione (GSH) and nitric oxide (NO) were determined using reagent kits obtained from Biodiagnostic Co., Egypt. Whereas liver lipid peroxidation level was estimated chemically according to the method described by Ruiz-Larrea *et al.* (1994) on the base of malondialdehyde (MDA) reaction with thiobarbituric acid (TBA) which forms a pink complex that can be measured photometrically.

#### **Histopathological Examination:**

General histological examination: The procedure for histological preparations is as described by Bancroft and Stevens (2013). Thyroid tissues were thinly sliced to a thickness of 3–4 mm, then fixed in 10% neutral buffered formalin (10% NBF), dehydrated in ethanol at varying

percentages, cleaned in xylene, and finally embedded in paraffin. To analyse overall tissue structure, the paraffin blocks were cut into sections using a microtome at a thickness of (4–6  $\mu$ m). Using a Leica microscope, H&E-stained slices were examined. (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland).

#### **Immunohistochemistry:**

##### Immunohistochemistry

Staining Protocol was carried out on paraffin sections and mounted on positively charged slides by using avidin-biotin-peroxidase complex (ABC) method (Hsu *et al.*, 1981). Anti-Rabbit Calcitonin Receptor Antibody (ThermoFisher Scientific, Cat# PA1-84457, Polyclonal, Dil.: 1:1000) was used in this IHC examination. Sections from each group were incubated with the previously mentioned antibodies, then the reagents required for the ABC method (Vectastain ABC-HRP kit, Vector laboratories) were added. Marker expression was labeled with peroxidase and colored with diaminobenzidine (DAB, produced by Sigma) to detect antigen-antibody complex. Negative controls were included using nonimmune serum in place of the primary or secondary antibodies. IHC stained sections were examined using a Leica microscope (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland) in Faculty of Veterinary Medicine, Cairo University.

#### **Statistical Analysis:**

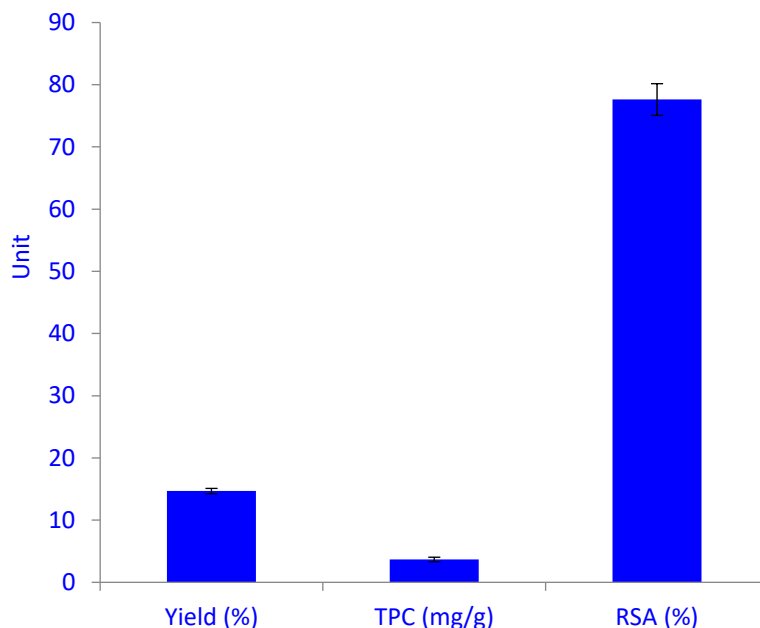
According to Steel and Torrie (1980), using a statistical analysis system (SAS) program software, the collected data were subjected to an ANOVA followed by Duncan multiple post hoc tests at the level of  $p \leq 0.05$ . Copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

#### **RESULTS**

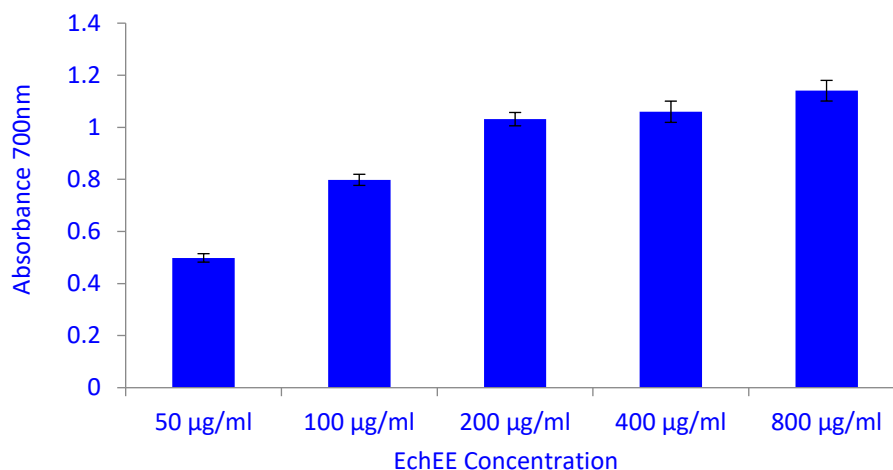
Results of *in vitro* assessments (yields, total phenolics content, radical scavenging activity and reducing power) of the EchEE are illustrated in Figures (1&2). It can be clearly noticed

that EchEE possesses considerable amounts of total phenolic compounds and higher antioxidant capacity achieved from the great ability to scavenge  $77.61 \pm 2.55$  % of DPPH' radical that confirms the resultant reducing power which reflected a concentration-dependent relation.

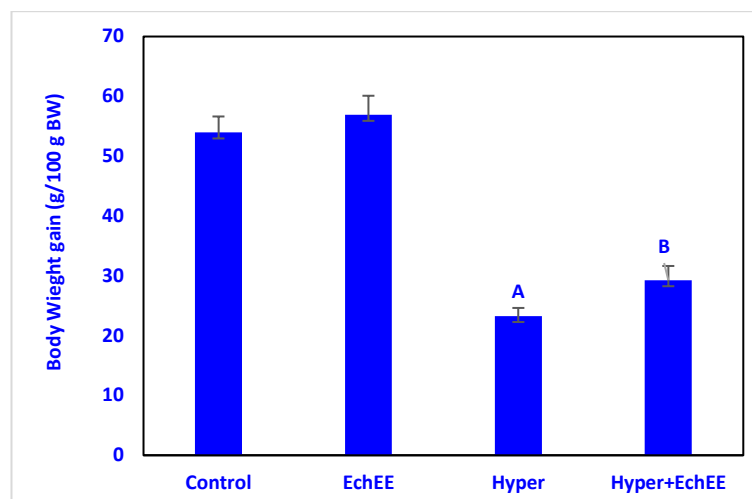
Ingestion of healthy rats with EchEE didn't affect the percent of body weight gain (BWG), while hyperthyroidism-modeled animals recorded a significant decrease in BWG when both groups were compared with the control group. Favorably, treatment of hyper-thyroidal animals with EchEE significantly restored BWG close to that of healthy animals (Fig. 3).



**Fig. 1.** The yield, total phenolics content (TPC) and radical scavenging activity (RSA) of *Echinacea purpurea* ethanolic extract (EchEE). Values are represented as mean  $\pm$  standard error for three replicates measurements.



**Fig. 2.** Reducing power ability of *Echinacea purpurea* ethanolic extract (EchEE). Values are represented as mean  $\pm$  standard error for three replicates measurements.



**Fig. 3.** Effect of *Echinacea purpurea* ethanolic extract (EchEE) on the rate of body weight gain (g/100 g b.w) of hyperthyroidism and treated animals' groups as compared to control one. Data are presented as mean  $\pm$  standard error for 10 rats. (<sup>A</sup>) is statistically significant from the control group while (<sup>B</sup>) is statistically significant from the hyperthyroid group at  $p \leq 0.05$ , EchEE (*Echinacea purpurea* ethanolic extract), Hyper (hyperthyroidism model).

The current study indicated that administration of EchEE to healthy animals didn't affect the hormonal profile of thyroid gland reflecting its safe effect on thyroid gland of healthy animals at the used level, while the serum level of total T3, total T4, free T3 and free T4 levels hormones was markedly increased matched with

noticeable decrease in TSH level of hyperthyroidism modeled animals. Interestingly, administration of hyperthyroidism-modeled animals with EchEE, resulted in a significant reduction in the serum total T3, total T4, free T3 and free T4 levels coupled with a remarkable elevation in TSH level (Table 1).

**Table 1.** Effect of *Echinacea purpurea* ethanolic extract (EchEE) on serum thyroid hormone profile of hyperthyroidism modeled animals.

	TSH	Total T3	Free T3	Total T4	Free T4
	ng/ml	ng/ml	pg/ml	µg/dl	ng/dl
Control	1.37 $\pm$ 0.21	72.8 $\pm$ 3.19	3.57 $\pm$ 0.04	6.44 $\pm$ 0.21	2.55 $\pm$ 0.34
EchEE	1.41 $\pm$ 0.08	69.9 $\pm$ 1.37	3.12 $\pm$ 0.03	6.11 $\pm$ 0.36	2.16 $\pm$ 0.12
Hyper	0.36 $\pm$ 0.05 <sup>A</sup>	211.4 $\pm$ 3.4 <sup>A</sup>	6.12 $\pm$ 0.11 <sup>A</sup>	18.27 $\pm$ 0.67 <sup>A</sup>	5.37 $\pm$ 0.49 <sup>A</sup>
Hyper +EchEE	0.78 $\pm$ 0.09 <sup>B</sup>	132.9 $\pm$ 3.1 <sup>B</sup>	4.11 $\pm$ 0.08 <sup>B</sup>	9.97 $\pm$ 0.37 <sup>B</sup>	3.75 $\pm$ 0.32 <sup>B</sup>

Data are presented as mean  $\pm$  standard error for 10 rats. (<sup>A</sup>) is statistically significant from control group while (<sup>B</sup>) is statistically significant from hyperthyroid group at  $p \leq 0.05$ , EchEE (*Echinacea purpurea* ethanolic extract), Hyper (hyperthyroidism model), TSH (thyroid stimulating hormone), T3 (triiodothyronine), T4 (thyroxine).

Administration of healthy animals with EchEE never disturb the values of Hb, fasting blood sugar (FBS), ASAT, ALAT, urea, and creatinine, indicating its nontoxic effect on liver and kidney; in contrast, hyperthyroidism led to a significant increase of serum ASAT and ALAT activity, coupled with

marked drop in Hb and FBS values; however, urea and creatinine levels insignificantly changed. Interestingly, treatment of hyperthyroidism group with EchEE significantly improved the values of Hb, FBS, ALAT, and ASAT near the control group (Table 2).

**Table 2.** Effect of *Echinacea purpurea* ethanolic extract (EchEE) on liver functions (ASAT and ALAT) and kidney functions (urea and creatinine) functions of hyperthyroidism modeled animals.

	<b>Hb</b> g/dl	<b>FBS</b> mg/dl	<b>ASAT</b> U/L	<b>ALAT</b> U/L	<b>Urea</b> mg/dl	<b>Creatinine</b> mg/dl
Control	15.2 ±1.29	97 ±4.2	30.16 ±4.8	33.73 ±2.6	42.71 ±3.8	1.12 ±0.08
EchEE	15.7 ±1.36	101 ±3.9	30.62 ±3.3	32.24 ±2.1	35.96 ±2.6	0.97 ±0.04
Hyper	9.33 ±1.24 <sup>A</sup>	76 ±3.5	38.90 ±2.8	42.28 ±2.4	40.10 ±1.7	1.05 ±0.07
Hyper +EchEE	13.11 ± 1.22 <sup>B</sup>	91 ±4.4	29.48 ±1.9	37.45 ±2.1	38.45 ±2.8	0.99 ±0.06

Data are presented as mean ± standard error for 10 rats. (<sup>A</sup>) is statistically significant from control group while (<sup>B</sup>) is statistically significant from hyperthyroid group at  $p \leq 0.05$ , EchEE (*Echinacea purpurea* ethanolic extract), Hyper (hyperthyroidism model),

Compared with the control group, hyperthyroidism induced marked reduction in hepatic GSH, GPx and SOD values matched with significant elevation in hepatic MDA and NO levels. Treatment of hyperthyroid animals with EchEE

showed significant restoration of the hepatic antioxidant markers (GSH, SOD and GPX), matched with remarkable downregulation in the oxidative markers (MDA and NO) (Table 3).

**Table 3.** Effect of *Echinacea purpurea* ethanolic extract (EchEE) on hepatic oxidative stress status markers of hyperthyroidism modeled animals.

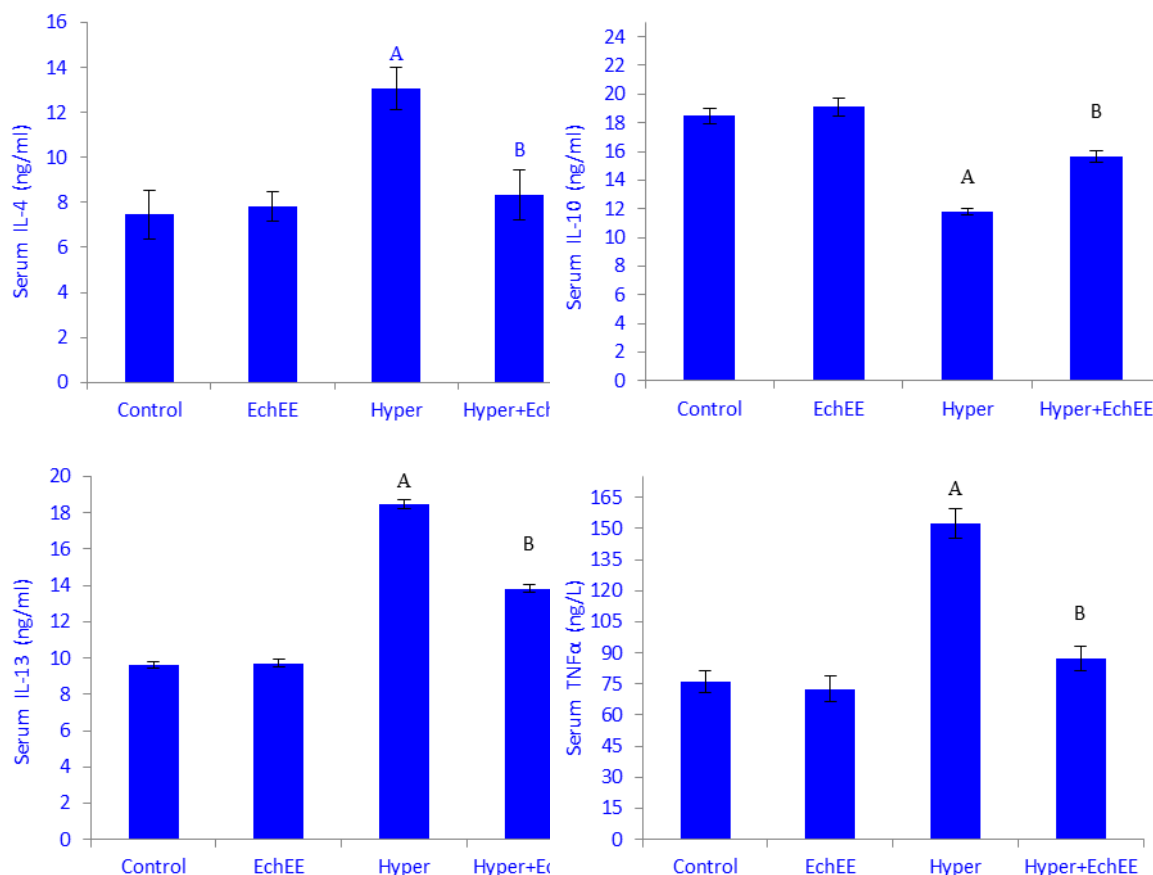
	<b>GPX</b> (U/g tissue)	<b>SOD</b> (U/g tissue)	<b>GSH</b> (mg/g tissue)	<b>NO</b> ( $\mu$ mol/g tissue)	<b>MDA</b> (nmol/g tissue)
Control	3092 ± 129	1598±37	2.13±0.08	10.3 ±0.99	63.2 ±3.21
EchEE	3114 ±133	1613±22	2.28 ±0.11	9.76 ±0.67	51.8 ±2.24
Hyper	2038 ±107 <sup>A</sup>	1033±28 <sup>A</sup>	0.79 ±0.033 <sup>A</sup>	22.3 ±1.18 <sup>A</sup>	154.3 ±5.55 <sup>A</sup>
Hyper +EchEE	2865 ±121 <sup>B</sup>	13878±42 <sup>B</sup>	1.92 ±0.09 <sup>B</sup>	13.4 ±1.11 <sup>B</sup>	89.6 ±4.08 <sup>B</sup>

Data are presented as mean ± standard error for 10 rats. (<sup>A</sup>) is statistically significant from control group while (<sup>B</sup>) is statistically significant from hyperthyroid group at  $p \leq 0.05$ , EchEE (*Echinacea purpurea* ethanolic extract), Hyper (hyperthyroidism model),

Significant increases in serum TNF $\alpha$ , IL-4, and IL-13 coupled with marked drop in IL-10 levels were detected regarding hyperthyroidism model. Favorably, hyperthyroid

animals that received EchEE revealed downregulation in TNF $\alpha$ , IL-4 and IL-13, associated with clear upregulation of the anti-inflammatory IL-10 close to the controls; (Fig. 5).





**Fig. 5.** Effect of *Echinacea purpurea* ethanolic extract (EchEE) on serum cytokine levels of hyperthyroidism modeled and treated rats. Data are presented as mean  $\pm$  standard error for 10 rats. (<sup>A</sup>) is statistically significant from the control group while (<sup>B</sup>) is statistically significant from hyperthyroid group at  $p \leq 0.05$ , EchEE (*Echinacea purpurea* ethanolic extract), Hyper (hyperthyroidism model).

#### Histopathological Examination:

Histological examination of thyroid sections Figure (6) from control rats detected the basic histological structure of thyroid follicles with their lining of simple cuboidal follicular epithelial cells which are characterized by eosinophilic cytoplasm as well as hyperchromatic spherical nuclei. Inside lumen of these follicles, a colloid is observed as consistent with pink color. In between thyroid follicles, polyhedral parafollicular cells in addition to intralobular fibrous connective tissue were noticed (A). Thyroid gland sections of *Echinacea purpurea* ethanolic extract group emphasized the same structure of thyroid follicles in negative control group involving; follicular epithelial cells, colloid, parafollicular cells as well as intra lobular fibrous connective tissue (B). Thyroid gland sections of

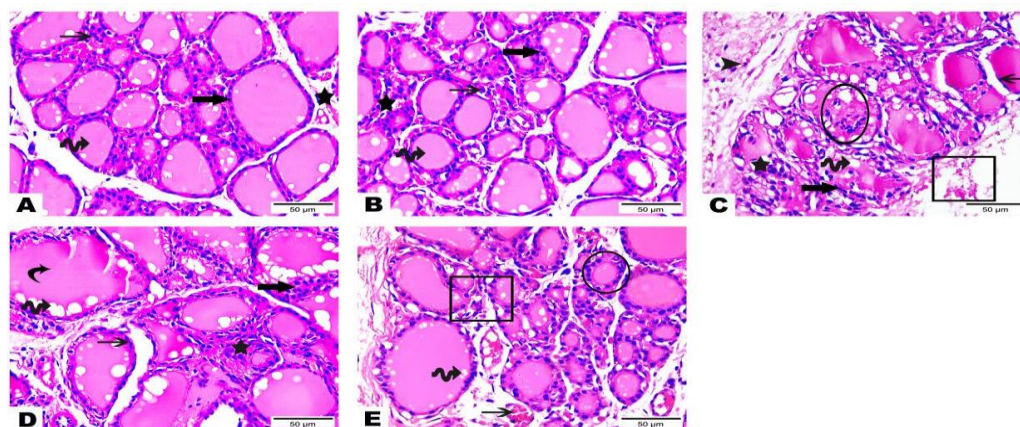
positive hyperthyroidism group underscored a variety of deteriorating changes along thyroid gland (C): Thyroid follicles presented with hyperplasia, flattening of epithelial follicular cells, detachment of basement membrane, epithelial desquamation, as well as scalloped colloids with a prominent expanding amount. Also, an obvious increase in parafollicular cells numbers, congested blood vessels, severe amount of fibrous connective tissue and leucocytic infiltration was detected (D). thyroid gland section of treated group revealed noticeable development in the thyroid gland structure. Most thyroid follicles existed with normal assembly of their follicular cells lining and slightly scalloped colloid. Diminished intralobular fibrous connective tissue along with

congested blood vessels were also observed (E).

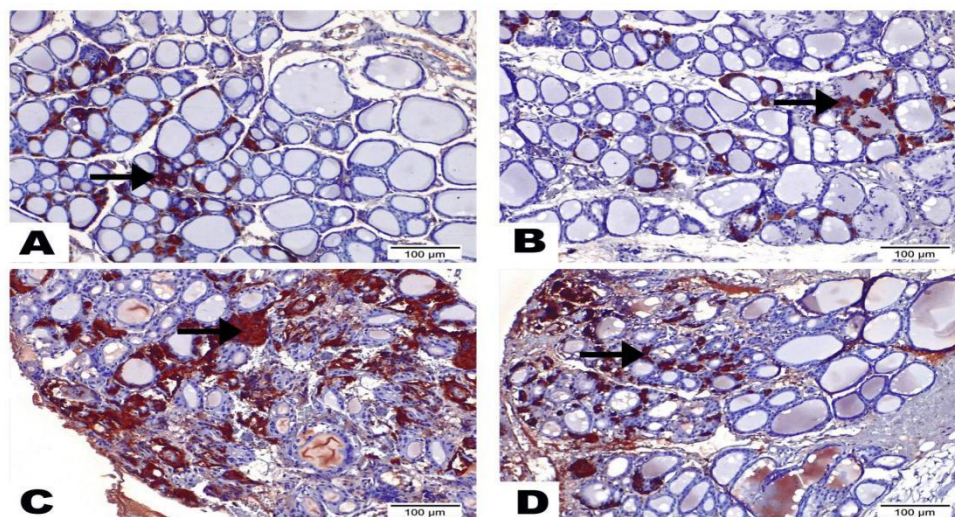
#### Immunohistochemical Findings:

Immunohistochemical examination demonstrated the reactivity of Calcitonin antibodies in thyroid gland tissue sections including researched groups (Figure 7). Thyroid gland section of the negative control group presented positive brown expression in a few amounts to Calcitonin antibody along parafollicular cells (A). Thyroid gland

section of *Echinacea purpurea* extract group exhibited slight expression of parafollicular cells to Calcitonin antibody like negative control group (B). Thyroid gland section of hyperthyroidism group underscored intense expression of Calcitonin antibodies along parafollicular cells as well as follicular ones (C). Thyroid gland section of treated group denoted moderate reactivity with brown positive expression to Calcitonin Antibody (D).



**Fig. 6.** Photomicrographs displayed the histopathological differences in thyroid gland tissue sections among analyzed groups as follows: (A) Thyroid gland sections of negative control group presented the basic histological structure of thyroid follicles with their lining of simple cuboidal follicular epithelial cells was characterized by eosinophilic cytoplasm as well as hyperchromatic spherical nuclei (thick arrow). Inside lumen of these follicles, colloid is observed as consistent with pink color (wave arrow). In between thyroid follicles, polyhedral parafollicular cells (thin arrow) in addition to intra-lobular fibrous connective tissue (star) were noticed. (B) Thyroid gland sections of EchEE group emphasized the same structure of thyroid follicles in negative control group involving; follicular epithelial cells (thick arrow), colloid (wave arrow), parafollicular cells (thin arrow) as well as intra lobular fibrous connective tissue (star). (C & D) Thyroid gland sections of Positive Hyperthyroidism Group underscored a variety of deteriorating changes along thyroid gland. (C): Thyroid follicles presented with hyperplasia (thick arrow), flattening of epithelial follicular cells, detachment of basement membrane (thin arrow), epithelial desquamation (circle), as well as scalloped colloid (wave arrow) with a prominent expanding amount (curved arrow). Also, an obvious increase in parafollicular cells numbers (star), congested blood vessels (cube), severe amount of fibrous connective tissue and leucocytic infiltration (arrowhead) were detected. (E) Thyroid gland section of treated Group revealed noticeable development in the thyroid gland structure. Most thyroid follicles existed with normal assembly of their follicular cells lining (circle) and slightly scalloped colloid (wave arrow). Diminished intralobular fibrous connective tissue (cube) along with congested blood vessels (thin arrow) were also observed. (Hematoxylin & Eosin Stain, Magnification Power= x400 & Scale Bar= 50 $\mu$ m).



**Fig. 7.** Photomicrographs demonstrated the reactivity of Calcitonin Antibody in thyroid gland tissue sections including researched groups as follows: (A) Thyroid gland section of negative control group presented positive brown expression in a few amounts to Calcitonin antibody (arrow) along parafollicular cells. (B) Thyroid gland section of EchEE Group exhibited slight expression of parafollicular cells to Calcitonin antibody (arrow) like negative control group. (C) Thyroid gland section of Hyperthyroidism Group underscored intense expression of Calcitonin Antibody (arrow) along parafollicular cells as well as follicular ones. (D) Thyroid gland section of treated group denoted moderate reactivity with brown positive expression to Calcitonin antibody (arrow). (Hematoxylin & Eosin Stain, Magnification Power= x200 & Scale Bar= 100µm).

### DISCUSSION

The hypermetabolic state resulting from hyperthyroidism is associated with various degrees of oxidative stress in the organism. Oxidative damage to lipids, proteins, and DNA, indicative of oxidative stress, was found in hyperthyroid rats (Duntas, 2002; Venditti and Meo, 2006). As the choice of anti-hyperthyroid drugs is not based on their relative treatment efficacy, but instead on consideration of side effects and risk of profound clinical consequences, in particular teratogenicity and serious injuries, therefore, the objective of the present study was to explore the possible modulatory potential of *Echinacea purpurea extract*, enhanced with its safe effect, on hyperthyroid modeled adult rats. The current study revealed that modeled hyperthyroidism was associated with a remarkable increase in serum total T3, FT3, total T4, and FT3 levels together with a notable drop in serum TSH level; In

addition, hyperthyroidism animals were asserted also by a decreased body weight gain. These results are consistent with many previous reports (Ourique *et al.*, 2013; Asker *et al.*, 2015; and Mannaa *et al.*, 2021).

The thyroid hormones significantly improved after EchEE-therapy in the hyperthyroidism-modeled rats, demonstrating the anti-hyperthyroidism potential of EchEE by balancing the levels of T3 and T4 and preventing the resulting loss in body weight.

Significant increases in serum TNF $\alpha$ , IL-4, and IL-13 coupled with significant decreases in IL-10 levels were detected regarding hyperthyroidism model, this result goes in line with that of Isaacs (1995) and Mannaa *et al.* (2021). Favorably, hyperthyroid animals that received EchEE revealed downregulation in TNF $\alpha$ , IL-4 and IL-13, associated with clear upregulation of the anti-inflammatory IL-10 close to the

controls; this result agonists the finding of Kishazi *et al.* (2018). It was reported that *Echinacea purpurea* extract is a promising immunomodulatory agent with a potent therapeutic value in stimulating the immune response (El-Sherniny *et al.*, 2021). Cytokines are glycosylated proteins that are involved in cell-cell communications and are often categorized as pro-inflammatory and anti-inflammatory. The anti-inflammatory IL-10 is well recognized as an important negative regulator of pro-inflammatory gene expression in mononuclear phagocytes; also, IL-10 is an immunosuppressive glycoprotein. It had been clear that IL-10 primarily acts on activated macrophages to suppress the secretion of IL-1, IL-12, TNF- $\alpha$ , and reactive oxygen radicals (Isaacs 1995).

Moreover, the hyperthyroidism model used in this study displayed a clear decrease in hepatic values of the antioxidative agents along with a marked elevation in the hepatic oxidative voltage. These deteriorations were improved by post-therapeutic doses of EchEE; this effect is evidenced by the marked elevation in the antioxidant markers (GSH, GPx, and SOD) coupled with significant reduction in the oxidative products (MDA and NO).

However, in the current study, the urea and creatinine levels unchanged in the hyperthyroid rat model. These findings are consistent with earlier studies that demonstrated that administering L-thyroxin did not affect serum urea and creatinine levels (Mannaa *et al.*, 2021).

Our findings regarding hyperthyroidism resulted in an increase in serum ASAT and ALAT activity, along with a significant decrease in Hb and FBS values. Previous studies have shown that hyperthyroidism causes the liver to undergo apoptosis, which leads to hepatic dysfunction (Huang and Liaw, 1995; Giris *et al.*, 2010), and is associated with an increase in liver enzymes like ALAT, ASAT, and

alkaline phosphatase in serum (Huang and Liaw, 1995; Mannaa *et al.*, 2021). Messarah *et al.* (2011) found that rats given L-thyroxine sodium salt (0.0012%) in drinking water had significantly lower levels of red blood cells, hemoglobin, and hematocrit.

The values of Hb and FBS considerably increased after EchEE treatment for the hyperthyroidism group. EchEE therapy improved liver enzymes and reduced oxidative stress, consequently, there is decreased leakage of hepatic ASAT and ALAT leakage into the bloodstream as EchEE contains antioxidant components like phenolic compounds that can suppress lipid peroxidation, stabilize cell membranes, and prevent the oxidation of membrane lipids, and restore the cellular integrity consequently.

By studying the thyroid gland tissue histopathologically, our biochemical findings were supported. The thyroid follicles displayed hyperplasia, flattening of the epithelial follicular cells, detachment of the basement membrane, desquamation of the epithelium, and scalloped colloid with a clearly increasing quantity. Additionally, there is a clear rise in the number of parafollicular cells, clogged blood arteries, a significant amount of fibrous connective tissue, and leucocytic infiltration. However, animals treated with EchEE resulted in an improvement in thyroid gland tissue as compared with the group of hyperthyroidisms, as EchEE indicated improvement in pathological changes. Previous research reported that vacuolated colloid was present inside the thyroid follicles of hyperthyroid rats. There was also an increase in interstitial tissue as well as the proliferation of bands of fibrous tissue and several layers of follicular cells. Reactive oxygen species, which are physiologically necessary for the synthesis of thyroid hormone and are produced in moderate amounts by thyroid epithelial cells in basal conditions, are continuously detoxified



either during the hormone-synthesis process or by endogenous antioxidant systems, making them non-toxic. (Mogulkoc *et al.*, 2005; Mannaa *et al.*, 2021). But when compared to the hyperthyroidism group, animals given EchEE showed improvement in thyroid gland tissue because EchEE showed improvement in the degenerative alterations. This improvement most likely resulted from reducing oxidative stress and improving thyroid hormones (Mannaa *et al.*, 2021). The thyroid gland tissue was found to be reactive to the Calcitonin Antibody by immunohistochemical analysis. The hyperthyroidism group's thyroid gland portion highlighted strong expression of calcitonin antibody along parafollicular cells. With brown positive expression to calcitonin antibody, the thyroid gland region of the EchEE Group indicated moderate reactivity.

### CONCLUSIONS

The outcomes of the present study showed that EchEE therapy efficiently regulated hyperthyroid hormones, improved liver and kidney functions and restored blood sugar and hemoglobin levels. The high concentration of antioxidant components in this extract may be responsible for its positive effects in treating hyperthyroidism and its accompanying problems. To support the significance of EchEE in treating human hyperthyroidism, more research is needed.

**Declaration of Interest:** The authors declare that there is no conflict of interest.

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## ARABIC SUMMARY

**الكفاءة المعدلة لمستخلص إشنسا بوربوريا على الفئران المصممة لفرط نشاط الغدة الدرقية**  
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**الخلفية والهدف:** تعتبر هرمونات الغدة الدرقية ضرورية للنمو، والتمثيل الغذائي، والتنظيم الحراري، والتطور. فرط نشاط الغدة الدرقية هو اضطراب في الغدة الدرقية يتميز بالإفراط في إفراز هرمونات الغدة الدرقية مما يؤدي إلى زيادة معدل التمثيل الغذائي واستخدام الأوكسجين في العديد من الأنسجة. إشنسا بوربوريا (EchEE) هو نبات طبي مهم له العديد من الفوائد الدوائية. **الهدف من العمل:** أجريت هذه الدراسة لتقييم الكفاءة المعدلة لمستخلص إشنسا بوربوريا الإيثانولي على الفئران المصممة لفرط نشاط الغدة الدرقية. **المواد والأساليب:** تم تصنيف كل من الحيوانات الطبيعية والحيوانات المصابة بفرط نشاط الغدة الدرقية إلى أربع مجموعات (10 فئران لكل منها (على النحو التالي) 1: حيوانات طبيعية بدون أي معالجات وعملت كعنصر تحكم طبيعي (2). الحيوانات الطبيعية التي يتم تناولها عن طريق الفم مع EchEE (465 مجم / كجم / يوم (مذابة في الماء لمدة 30 يوماً) ؛ 3 الحيوانات التي تشبه فرط نشاط الغدة الدرقية غير المعالجة ؛ (5 حيوانات على غرار فرط نشاط الغدة الدرقية عولجت ب EchEE لفترة مماثلة. **النتائج:** أدى علاج الحيوانات المصممة لفرط نشاط الغدة الدرقية باستخدام EchEE إلى انخفاض كبير في مستوى المصل من إجمالي T3 وإجمالي T4 و T3 الحر و T4 الحر إلى جانب ارتفاع ملحوظ في مستوى TSH. نجح EchEE أيضاً في تحسين الصورة النسيجية والكيميائية المناعية للغدة الدرقية. أيضاً، مما أدى إلى تحسين وظائف الكبد والكلية وانخفاض مستويات السكر في الدم. **الاستنتاجات:** خلصت هذه الدراسة إلى أن EchEE حسّن بشكل فعال البنية الفيزيولوجية المرضية للغدة المفرطة الدرقية، وقد تحقق ذلك من الانخفاض الملحوظ في هرمونها مع زيادة ملحوظة في TSH. يمكن ميكنة هذا التأثير من خلال الخصائص المضادة للأكسدة لمكونات EchEE. يمكن أن يكون هذا المستخلص عاملاً طبيًا واعدًا لصحة الإنسان؛ هناك حاجة إلى مزيد من البحث.

**الكلمات المفتاحية:** فرط نشاط الغدة الدرقية، هرمونات الغدة الدرقية ، إشنسا بوربوريا ، مضادات الأكسدة ، أمراض الهيستوباثولوجيا المناعية ، الجرذان.