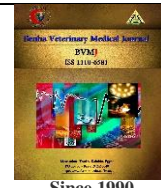




Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



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Original Paper

Spirulina platensis and Grape Seed Proanthocyanidin Extract ameliorates hepatic impairment in Carbimazole-induced hypothyroidism in male rats via caspase 8/Bcl-2 signaling pathway

Samy A. Hussein¹, Samir A. Abdel- Aal², Mohammed A. Marzouk¹, Marwa Darweish³, Asmaa H. A. Hussain¹

¹ Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt.

² Department of Animal, poultry and environmental hygiene, Faculty of Veterinary Medicine, Benha University, Egypt.

³ Department of Pathology, Faculty of Veterinary Medicine, Benha University, Egypt.

ARTICLE INFO

Keywords

Hypothyroidism
GSPE
Spirulina
Thyroid hormones
Apoptosis

Received 15/07/2022

Accepted 06/09/2022

Available On-Line
01/10/2022

ABSTRACT

Spirulina and Grape Seed Proanthocyanidin Extract (GSPE) were studied for their potential therapeutic and protective properties in hypothyroidism-induced rats. Six equal groups of forty-eight rats were formed. Group 1 is a control and Group 2 (hypothyroidism) received an oral dose of carbimazole (1.8 mg/kg b.wt.) every day. Rats in Group 3 (GSPE Protected) got (150/kg b. wt) of GSPE per day for the first 3 weeks, then continued administered with carbimazole for another 3 weeks. Group 4 (carbimazole + GSPE treated): Rats were administered a daily dose of carbimazole (1.8mg/kg b.wt) for 3 weeks, followed by GPSE (150/kg b. wt/day) for another 3 weeks. Group 5 (Spirulina Protected); Rats received Spirulina (300 mg/kg body weight per day) for the first three weeks, then continued with carbimazole for another 3 weeks. Group 6 (Spirulina treated): Rats administered a daily dose of carbimazole for 3 weeks, followed by Spirulina (300/kg b. wt/day) for other 3 weeks. The results collected demonstrated a significant rise in liver Caspase-8 gene expression in the hypothyroidism group along with significant increases in blood TSH, total cholesterol, and triacylglycerols. However, serum T3, T4 levels drastically dropped along with Bcl2 expression in the liver tissue. Treatment and protection of spirulina and GPSE reduced TSH, total cholesterol, triacylglycerols and Caspase-8 with significant upregulation of Bcl-2 and marked decrease in total cholesterol and triacylglycerols concentrations.

In conclusion, grape seed extracts and Spirulina platensis had therapeutic potential in hypothyroidism, protect liver damage and mitigate Thyroid disruptors via anti-apoptotic activities.

1. INTRODUCTION

The thyroid gland is where the two iodine-containing amine hormones known as thyroid hormones—derived from the amino acid tyrosine—are synthesized. Thyroid stimulating hormone (TSH) is released by the anterior pituitary in response to thyroid releasing hormone (TRH), which in turn stimulates the thyroid follicular cells to release T4 (80%) and T3 (20%) (Khanam, 2017). Thyroid hormones control several metabolic pathways that are involved in the breakdown of proteins, lipids, and carbohydrates. Also, these hormones control bile acid production, fatty acid metabolism, and cholesterol homeostasis in the liver (Mullur et al; 2014). Notably, hypothyroidism is characterized by increased cholesterol levels, reduced lipolysis, and gluconeogenesis (Cicatiello et al; 2018). L-thyroxine and carbimazole, two medications used to treat thyroid conditions, are also used in animal experiments to cause hypothyroidism and hyperthyroidism (Treesh and Khair, 2014). Hypothyroidism is brought on by a TSH deficiency or absence. It is one of the most prevalent thyroid conditions in people, and it can be either congenital or acquired (Ayuob 2016). It can be brought on by malfunctions in the processes

that regulate the production of thyroid hormones or by issues that develop during the course of hyperthyroidism treatment (Hashem et al; 2016). All bodily processes slowdown in the hypothyroid state, which is a complicated hormonal imbalance (Hayat et al; 2010) Reduced basal metabolic rate and increased generation of reactive oxygen species (ROS) are both symptoms of hypothyroidism (Rabehand El-Ghandour, 2016). Metabolic depression resulting from hypothyroidism has been associated with a decrease in oxidant production (Işman et al; 2003). Hypothyroidism was induced by the anti-thyroid drug carbimazole, which is used in the treatment of human hyperthyroidism. (Deshpande et al., 2002). SP is regarded as a top-notch dietary supplement with numerous health advantages. There are plenty of proteins, carotenoid (β -Carotene), polyunsaturated fatty acids, glycolipids, polysaccharides, vitamins A, E, and B, as well as iodine, calcium, magnesium, manganese, potassium, zinc, and iron (Hoseini et al; 2013). Additionally, phycocyanin, a potent antioxidant that gives spirulina its deep green colour, is found in SP (Lissi et al; 2000). SP is well documented for its clinical importance in diabetes, hypertension, and cancer (Palaniswamy and Veluchamy, 2018), besides its antioxidant, immune-

* Corresponding author: elasal_81@yahoo.com

modulating, anti-microbial (Finamore et al; 2017), and radio-protective properties (Ibrahim, 2012, 2014). Many woody plants, including grape seeds and white pine, contain pro-anthocyanidins (PAs), oligomers and polymers of monomeric flavonoids. In comparison to vitamins C, E, and -Carotene, pro-anthocyanidins (Pas) contained in grape seed extract (GSE) exhibit much better protection against oxygen free radicals and have a wide range of biological, pharmacological, and chemo-protective activities (Bagchi et al; 2002; Sen and Bagchi, 2001). Studies have demonstrated that PAs can reduce drug-induced liver and kidney damage and have strong antioxidant and anticancer action by scavenging superoxide and hydroxyl radicals (Da Silva, 1991; Engelbrecht et al; 2007). Therefore, the goal of the current investigation was to determine if *Spirulina platensis* and grape seed pro-anthocyanidin extract could reduce hepatic dysfunction and thyroid disruptors in rats with carbimazole-induced hypothyroidism.

2. MATERIAL AND METHODS

2.1 Experimental Animals

Forty-eight male rats, aged 8–12 weeks old and weighing about 140–160 g, were used in this study. Throughout the course of the experiment, rats were kept in separate metal cages under constant environmental and dietary circumstances. The rats were given a consistent ration of food, and unlimited access to fresh, clean drinking water was provided. Prior to the start of the trial, all rats underwent an acclimatisation phase lasting a minimum of 15 days. The Benha University Animal Care and Use Committee authorised the experimental protocols, which adhere to the National Institutes of Health's guide for the care and use of laboratory animals.

2.2 Chemical and Antioxidant agents:

2.2.1 Carbimazole (CMZ)(NeoMercazole)[®]:

NeoMercazole[®] 5 mg pills, the brand name for carbimazole, were produced by Amdipharm in Dublin, Ireland. Carbimazole was administered orally to a hypothyroid rat model at a dose of (1.8 mg/kg b.wt/day) for a total of three weeks (Sakr et al; 2012).

2.2.2 *Spirulina platensis*

The source of pure *Spirulina platensis* powder was (National Research Center- Dokki-Egypt). Dosage: Rats received 1.0 mL of a distilled water suspension containing 300 mg/kg body weight of spray-dried *spirulina platensis* powder (Simsek et al; 2009).

2.2.3 Grape Seed Proanthocyanidin Extract (GSPE)

The supplier of (GSPE) (Al Debeiky Pharma Company for Pharmaceutical industries, Al Obour, Cairo, Egypt). The chosen dose of GSPE was (150 mg/kg b.wt) (Yamakoshi et al., 2002) and (Yousef et al., 2009). DMSO 7% was used to dissolve the GSPE, and sterile saline solution was used to dilute it to the proper concentration (Mona A. El-Gawish et al.; 2006).

2.3 Experiment Design

After two weeks of acclimatization, the rats were randomly assigned to 6 equal groups of 8 rats each.

G1 (Normal): rats were kept without medication.

G2 (hypothyroidism): Rats received Carbimazole (1.8mg/kg b.wt/day) orally for 3 weeks.

G3 (GSPE + hypothyroid Protected): for the first three weeks, rats received GSPE (150/kg body weight/day), which was then followed with Carbimazole (1.8mg/kg body weight) for an additional three weeks.

G4 (GSPE+ hypothyroid treated): Carbimazole (1.8 mg/kg b.wt/day) was given to rats for 3 weeks, followed by GPSE (150 mg/kg b.wt/day) for an additional 3 weeks.

G5 (Spirulina + hypothyroid Protected); For the first three weeks, rats were given spirulina (300 mg/kg b.wt./day), and for the next three weeks, Carbimazole (1.8 mg/kg b.wt.).

G6 (Spirulina + hypothyroid treated): Carbimazole (1.8 mg/kg b.wt/day) was given to rats for 3 weeks, followed by Spirulina (300 mg/kg b.wt/day) for an additional 3 weeks.

2.4 Sampling

Ocular vein punctures were used to collect blood samples for serum separation at the conclusion of each experimental period and after an overnight fast. The blood samples were then placed in dry, clean, screw-capped tubes, and the serum was separated by centrifugation at 2500 rpm for 15 minutes. When ready for use in a subsequent biochemical study, the clear, clean serum was preserved in a deep freezer at -20 o C after being separated by an automatic pipette and received in a dry, sterile samples tube. All sera underwent the following analyses:

Triacylglycerols, Triiodothyronine (T3), Thyroxin (T4), Total Cholesterol, and Thyroid Stimulating Hormone (TSH).

2.4.2. Tissue specimens

Rats were sacrificed by decapitation in accordance with animal ethics committees at the conclusion of the experiment (6 weeks), after which the abdomen was opened and hearts were retrieved.

A- For molecular analysis:

The liver tissues were collected from all animals' groups, put in Eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of Caspase 8 and Bcl-2 gene expression and DNA damage by comet assay.

B-For Histopathological examination (thyroid and liver):

Small tissue specimens from thyroid and liver tissue were obtained, quickly preserved in 10% neutral buffered formalin solution, and then then thin paraffin sections were papered and stained by H&E stain (Dray and Walling, 1976).

2.5. Analysis:

2.5.1 Biochemical analysis

Serum total cholesterol and triacylglycerols levels were estimated using a method of Ellefson and Caraway, (1976), respectively. Serum TSH and T4 concentrations were Quantitatively determined by a solid phase enzyme immunoassay using ELISA kit (XEMA.Co) with a catalog No.: K2011 and K212I, respectively. However, serum T3 was done by an Electrochemiluminescence (ECLIA) immunoassay and carried out using (Cobase 801 immunoassay analyzer) with code number: 10032.

2.5.2 Molecular analysis (Liver)

Real-time qPCR analysis was used to assess the Caspase-8 and Bcl-2 mRNA expression in rat liver. The load was managed with -actin. According to the manufacturer's recommendations, total RNA was extracted from the liver using the High Kit for extraction of pure RNA (Thermo Scientific, Fermentas, #K0731) RNA Extraction kit. RevertAid TM First Strand cDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA) was used to reverse transcribe each cDNA sample. Then, using the Faststart

Universal SYBR Green Master, real-time quantitative PCR amplification was carried out (Roche, GER). Using the 2-Ct

technique, the target gene was normalised with β -actin (Livak and Schmittgen, 2001) (Table A).

Table A Forward and reverse primers sequence for primers used in qPCR.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
Caspase 8	CTGGGAAGGATCGACGATTA	CATGTCCTGCATTTTGATGG
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
B-actin	AAGTCCCTCACCTCCCAAAAG	AAGCAATGCTGTACCTTCCC

2.5.3 DNA damage analysis (Comet assay):

After various treatments, the comet test was used to check for any potential DNA damage. The movement of DNA from immobilized nuclear DNA is measured to identify DNA strand breaks and alkali labile spots. The comet assay was carried out in the current investigation in accordance with the instructions provided by Singh et al, (1988).

2.5.4 Statistical analysis:

All the data were provided as (Mean ± S.E). Duncan's multiple range test was used to produce individual comparisons, and one-way analysis of variance (ANOVA) was used to assess statistical significance using SPSS 18.0 software in 2011. (DMRT). The differences were considered statistically significant when the value of p was ≤ 0.05.

3. RESULTS

Comparing with control normal group, carbimazole-induced hypothyroidism was associated with a considerable rise in serum TSH. However, the group that received carbimazole was found to have significantly lower serum T3 and T4

concentrations. TSH was significantly lower and blood T3 and T4 levels were noticeably higher after treatment and protection with GSPE (G4) and spirulina (G6) than in the carbimazole-induced hypothyroidism group (Table 1 and Figures 1, 2, 3). When compared to the normal control group, a substantial rise in the concentrations of triacylglycerol and total cholesterol in the serum was seen in the carbimazole-induced hypothyroidism group. Rats given GSPE (G3 and G4) or spirulina (G5 and G6) treatment or protection from carbimazole-induced hypothyroidism showed a considerable drop in serum total cholesterol and triglycerides levels compared to the hypothyroidism group (Table 2 and Figures 4, 5).

A significant upregulation in Caspase8 and downregulation in Bcl-2 were observed in Carbimazole induced hypothyroidism group. Treatment and protection with GSPE (G3 and G4), spirulina (G5 and G6) to hypothyroid rats exhibited a significant downregulation in Caspase8 with upregulation in Bcl-2 as compared with hypothyroid non treated group (Table 3 and Figures 6, 7).

Table 1 Effect of protection and treatment with Spirulina or GSPE on serum TSH, T3 and T4 concentrations of Carbimazole induced hypothyroidism in rats.

Animal groups	TSH (nU/ml)	T3 (pg/ml)	T4 (pg/ml)
Control non treated (G1)	4.34±0.14 ^c	4.42 ± 0.10 ^a	6.34 ± 0.17 ^a
Hypothyroidism (G2)	5.93± 0.15 ^a	2.54 ± 0.10 ^c	4.12 ± 0.15 ^c
Hypothyroidism+ GSPE protection (G3)	4.57±0.10 ^c	4.02 ± 0.14 ^b	6.02 ± 0.14 ^a
Hypothyroidism + GSPE treatment (G4)	5.27±0.14 ^b	3.14 ± 0.10 ^d	5.14 ± 0.15 ^b
Hypothyroid+ spirulina protection (G5)	4.42±0.13 ^c	4.30 ± 0.13 ^a	6.11 ± 0.20 ^a
Hypothyroidism+ spirulina treatment(G6)	5.12±0.13 ^b	3.50 ± 0.11 ^c	5.20 ± 0.15 ^b

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

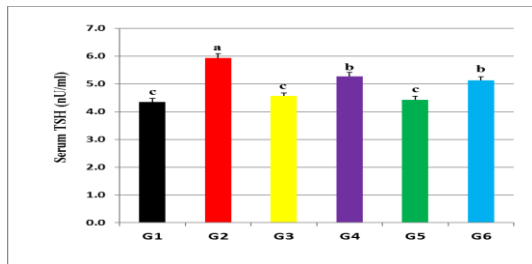


Fig (1) Effect of Spirulina or GSPE treatment on serum TSH concentration in experimental model of hypothyroidism in rats.

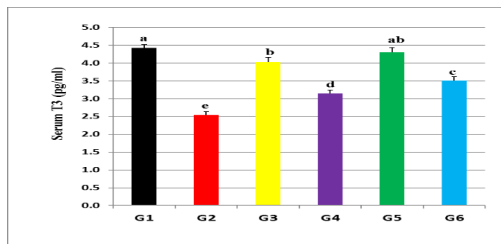


Fig. (2) Effect of Spirulina or GSPE treatment on serum T3 concentration in experimental model of hypothyroidism in rats

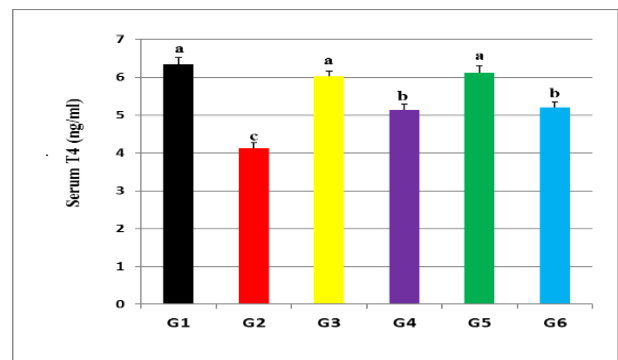


Fig (3) Effect of Spirulina or GSPE treatment on serum T4 concentration in experimental model of hypothyroidism in rats.

Table 2 Effect of protection and treatment with Spirulina or GSPE on serum total cholesterol and triacylglycerol concentrations of Carbimazole induced hypothyroidism in rats.

Animal groups	Total Cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Control non treated(G1)	94.17 ± 2.02e	78.56 ±1.62e
Hypothyroidism(G2)	154.38 ± 4.25a	138.14 ±2.76a
Hypothyroidism+ GSPE protection(G3)	108.06 ± 2.19c	100.24 ±1.78d
Hypothyroidism + GSPE treatment(G4)	130.11 ± 3.18b	120.47 ±2.40b
Hypothyroid+ spirulina protection(G5)	100.26 ± 1.93d	93.19 ±1.73d
Hypothyroidism+ spirulina treatment(G6)	112.36 ± 2.67c	109.51 ± 2.16c

Data are presented as (Mean ±S.E). SE = Standard error. Mean values with different superscript letters in the same column are significantly different at(P≤0.05).

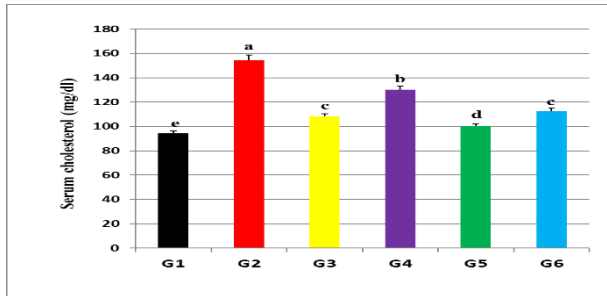


Fig (4) Effect of Spirulina or GSPE treatment on serum total cholesterol concentration in experimental model of hypothyroidism in rats.

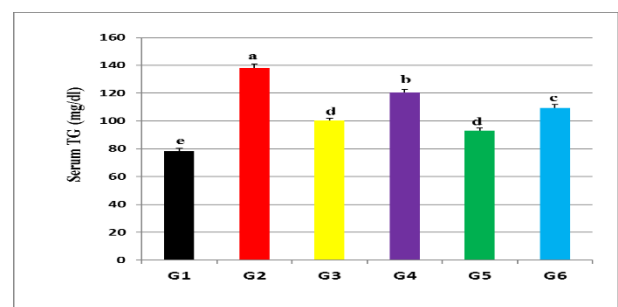


Fig (5) Effect of Spirulina or GSPE treatment on serum triacylglycerol concentration in experimental model of hypothyroidism in rats.

Table 3 Effect of protection and treatment with Spirulina or GSPE on liver Caspase 8 and Bcl-2 gene expression of Carbimazole induced hypothyroidism in rats.

Animal Groups	Caspase 8 Fold change±SEM	Bcl-2 Fold change±SEM
Control non treated(G1)	1.00 ± 0.08 ^f	1.00 ±0.05 ^a
Hypothyroidism(G2)	8.88 ± 0.37 ^a	0.01 ±0.005 ^e
Hypothyroidism+ GSPE protection(G3)	4.56 ± 0.29 ^d	0.14 ±0.02 ^c
Hypothyroidism + GSPE treatment(G4)	7.26 ± 0.31 ^b	0.06 ±.01 ^d
Hypothyroid+ spirulina protection(G5)	1.74 ± 0.09 ^e	0.30 ±0.02 ^b
Hypothyroidism+ spirulina treatment(G6)	6.11 ± 0.32 ^c	0.12 ±0.01 ^c

SEM, Standard Error of Mean. Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

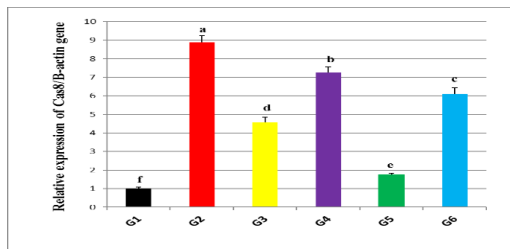


Fig (6) Effect of Spirulina or GSPE treatment on liver caspase8 gene expression of experimental model of hypothyroidism in rats.

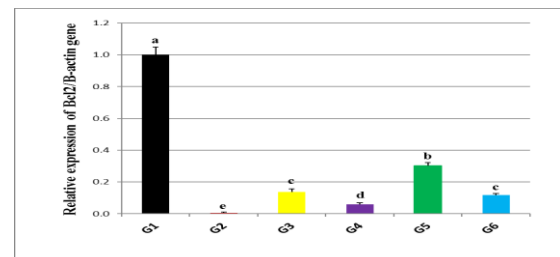


Fig (7) Effect of Spirulina or GSPE treatment on liver Bcl-2 gene expression of experimental model of hypothyroidism in rats.

Comet assay (DNA damage in liver tissue)

DNA damage in the rat liver following administration of Carbimazole and the treatment and the protective effect of Spirulina or Grape seed administrations was evaluated by comet assay. The comet assay results, which are shown in Table (4) and Figure (8), demonstrated that carbimazole administration significantly increased DNA damage, as

evidenced by an increase in tail length and tail DNA percentage in liver tissue from rats with hypothyroidism as compared to the normal control group. Compared to the hypothyroidism untreated group, DNA damage was dramatically reduced in the Spirulina or Grape seed treated groups. Furthermore, a significant decrease in DNA damage was noticed in G4 and the highest decrease in G5.

Table 4 Comet assay parameters obtained by image analysis of rat liver in Carbimazole administered rats following treatment with Spirulina or GSPE:

Animal group	Tailed (%)	Untailed (%)	Tails length (µm)	Tail DNA (%)	Tail moment
Control non treated(G1)	2.5	97.5	1.22±0.11 ^f	1.41	1.72
Hypothyroidism(G2)	33	67	10.02±0.53 ^a	8.90	89.58
Hypothyroidism+ GSPE protection(G3)	13	87	4.40±0.19 ^d	4.29	18.88
Hypothyroidism + GSPE treatment(G4)	24	76	7.31±0.47 ^b	6.03	44.08
Hypothyroid+ spirulina protection(G5)	9	91	2.98±0.12 ^e	3.41	10.16
Hypothyroidism+ spirulina treatment(G6)	17	83	6.05±0.28 ^c	4.75	28.73

Means within the same column of tail length carrying different superscript letters are significantly different (P≤ 0.05).

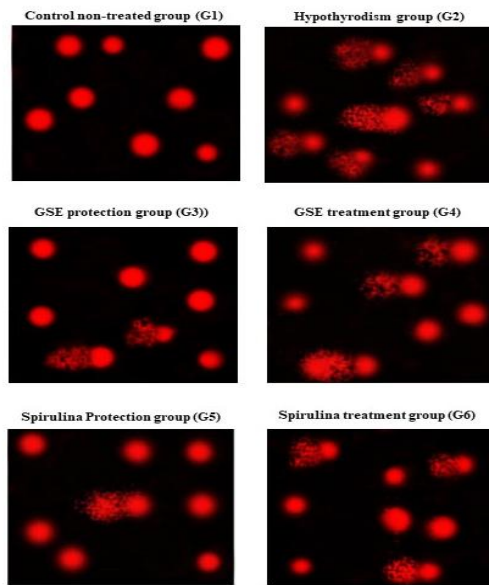


Fig (8) Photomicrographs representation of liver DNA damage, using comet assay, in Carbimazole administered rats following treatment with Spirulina or GSPE

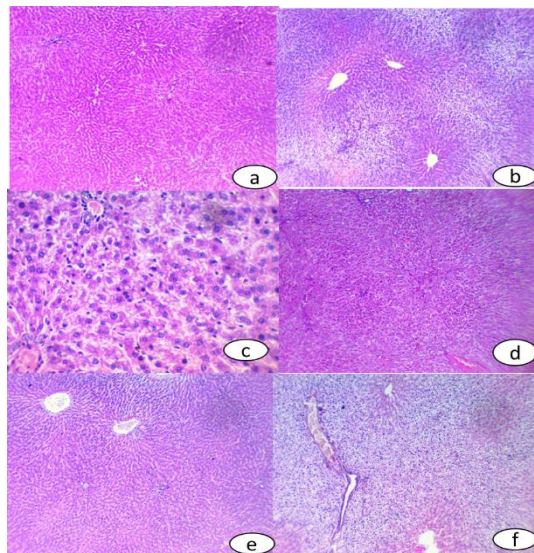


Fig (9) Histopathological finding of thyroid gland in control group a: showed low cuboidal epithelium with homogenous eosinophilic secretion. Hypothyroidism group b: showed hyper cellularity of cuboidal epithelium (arrow). Spirulina protected group c: showed cuboidal epithelium with faint eosinophilic secretion in some follicles with other showed hyperplasia of lining epithelium of glands (star) while some follicular epithelium suffered from vacuolar degeneration (arrow). Spirulina treated group d: showed mostly normal follicles while some showed flattened epithelium (arrow). Grape seed protected group e: showed thyroid follicle eosinophilic secretion with vesicular nucleus. Grape seed treated group showed follicles with hyperplasia of interstitial epithelium (arrow). H&E 400 x

4.DISCUSSION

Growth, neural development, reproduction, and energy consumption require thyroid hormones. Hypothyroidism and hyperthyroidism are global health problems. Iodine diet is a key risk factor for thyroid illness, but other factors such as age, smoking status, genetic susceptibility, ethnicity, endocrine disruptors, and novel therapies, such as immune checkpoint inhibitors, also have an impact (Chiovato et al; 2019). Many studies reported that Carbimazole is an anti-thyroid drug (ATD) that belongs to the thalidomide class. It is a medical treatment option for Graves' disease (GD) (Kheder et al. 2018). Carbimazole and its active metabolite methimazole are the first-line treatments for GD

Histopathological finding of thyroid gland in control group showed thyroid follicles lined with cuboidal epithelium with homogenous eosinophilic secretion in their lumens. In hypothyroidism group thyroid gland showed hyper cellularity of cuboidal epithelium while spirulina protected group showed cuboidal epithelium with faint eosinophilic secretion in some follicles with other showed hyperplasia of lining epithelium of glands while some follicular epithelium suffered from vacuolar degeneration. Spirulina treated group showed mostly normal follicles while some showed flattened epithelium. grape seed protected showed eosinophilic secretion with vesicular nucleus. The examined thyroid gland in grape seed treated group showed follicles with hyperplasia of interstitial epithelium (Fig., 9). Moreover, the control group's liver displayed normal hepatic architecture, while examined in hypothyroidism group showed periphero-lobular hydropic degeneration. Spirulina protected group showed congestion of central veins with leucocytic infiltration, while hepatocytes suffered from vacuolar degeneration. Spirulina treated group showed mostly normal hepatic architecture. Moreover, grape seed protected showed normal hepatic lobules and the liver of rats in grape seed treated group showed diffuse hydropic degeneration (Fig.,10).

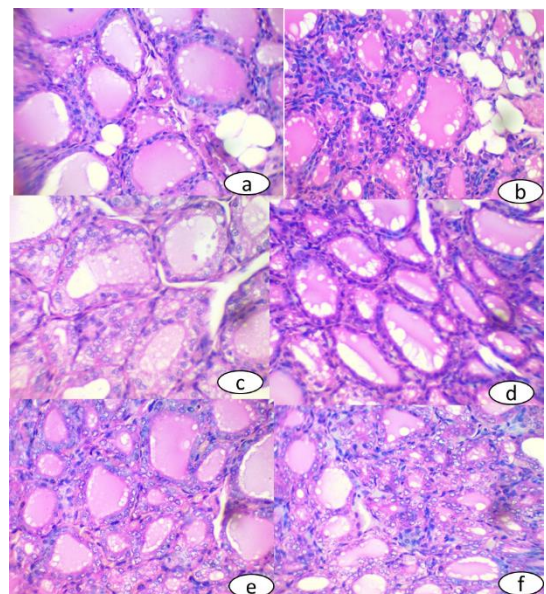


Fig (10) Histopathological finding of liver of control group a: showed normal hepatic architecture H&E 100 x. Hypothyroidism group b: showed preperilo-lobular hydropic degeneration H&E 100 x. Spirulina protected group c: showed congestion of central vein with leucocytic infiltration (arrow), while hepatocytes suffered from vacuolar degeneration H&E 400 x. Spirulina treated group d: showed mostly normal hepatic architecture H&E 100 x. Grape seed protected group e: showed normal hepatic lobules H&E 100 x. Grape seed treated group showed diffuse hydropic degeneration H&E 100.

(Norsworthy et al. 2022). Carbimazole inhibits thyroid peroxidase (TPO) and decreases iodide incorporation into tyrosine molecules. It also prevents the formation of T4 and T3 from mono- and di-iodinated residues. Carbimazole has been the drug of choice in some hyperthyroid patients because it has fewer side effects, such as gastrointestinal problems (Hammad and Abdelgadir, 2021). Carbimazole caused a considerable rise in serum TSH. (CMZ) induced. However, in hypothyroid rats, serum T3 and T4 concentrations were significantly reduced. Similarly, EL-Ghareeb et al. (2016) mentioned that throughout the first two to three weeks of postnatal life, when the cerebellum was still developing, it was noted that CMZ injection

caused a considerable rise in blood TSH levels in rats. Also, Al-Naely et al. (2018) found that Carbimazole administered for 45 days exhibited an increase in TSH level. Many studies reported the elevation of TSH and decreasing of T4 & T3 by administration of CMZ (Ibrahim et al. 2021). Likewise, Ahmed and Abd El Tawab (2010) reported that Methimazole (MMI) Anti-thyroid medication prevents the coupling of iodotyrosyl residues to generate iodothyronine and interferes with the incorporation of iodine into the tyrosyl residues of thyroglobulin, preventing the production of thyroid hormones T3 and T4. Because carbimazole is known to induce hypothyroidism by blocking the thyroid peroxidase enzyme, treatment of adult female rats dramatically reduced the serum levels of T3 and T4 in both mothers and their offspring while increasing the serum TSH level, which plays a key role in the production of thyroid hormones that carry out the oxidative coupling of iodinated tyrosine and the iodination of tyrosine residues in thyroglobulin (Ilyas et al. 2015).

In the current study GSPE or spirulina treatment of hypothyroidism showed a large drop in TSH and a noticeable rise in serum T3 and T4 concentrations. This result was supported by Martins et al., (2021) who attributed this to the anti-apoptotic, anti-necrotic, anti-inflammatory, and antioxidant properties of GSPE. The presence of phenolic compounds, particularly proanthocyanidins, which have a higher antioxidant capacity than vitamin C or E, contributes to its ability to scavenge oxidants and free radicals. Omar et al. (2022) suggested that administration of spirulina orally considerably increased the serum levels of T3 and T4 in humans. Also, Martins et al. (2021) proven the TSH lowering effect of Spirulina in rats with hypothyroidism. In the present study, the improvement of thyroid function tests after treatment, established the anti-hypothyroid activity of GSPE and Spirulina. Moreover, Al-Naely et al. (2018) reported that the significantly enhanced activities of GSE at 150 mg/kg body weight for 2 weeks scientifically suggest that the extract of Grape seed has the potential resistance against the negative role of CMZ enhancing the factors that produce T4 and T3. The containing of the flavonoids may explain the reasons after the elevation of T4 over T3 in liver, is the potential of inhibiting the type 1 iodothyronine deiodinase (D1) is an essential enzyme which converts the prohormone T4 into active T3 in liver. Also, Martins et al. (2021) establish the significance enhancement of T3 and T4 after supplementation of 400 mg/kg body weight of GSE.

Carbimazole-induced hypothyroidism was associated with a considerable increase in the serum total cholesterol and triacylglycerol levels. Similarly, Al-Naely et al. (2018) reported that carbimazole causes a decrease in thyroid hormones, which can regulate fat metabolism and lipid levels. As a result, a decrease in thyroid hormone leads to high cholesterol due to its control over cholesterol synthesis via gene expression and mRNA reproduction. Who added that, thyroid hormone deficiency causes an imbalance in fat digestion, absorption, synthesis, or prevents activation of their receptors. So, hypothyroidism reduces the activity of Lipoprotein lipase in fatty tissues and liver cells, which is responsible for fat metabolism, resulting in a high concentration of TG, HDL, and vLDL.

In the current study serum total cholesterol and triacylglycerols concentrations were markedly decrease in GSPE or spirulina treated hypothyroid rats. Proanthocyanidins, one of the grape compounds, played a role in lowering lipid concentration by promoting cholesterol transfer and increasing the effectiveness of its secretion by

bile. Polyphenolic compounds in GSE, such as gallic acid, catechin, and epicatechin, have been shown to lower cholesterol by inhibiting the pancreatic cholesterol esterase enzyme, which hydrolyzes cholesterol in food and facilitates solubility in colloidal micelles to facilitate absorption, resulting in lower cholesterol absorption into the bloodstream. These compounds also stimulate the synthesis of bile acid from cholesterol (Peters et al. 2019). Moreover, Serban et al. (2016) found that spirulina supplementation increased levels of HDL cholesterol while lowering levels of total cholesterol, LDL cholesterol, and triglycerides in plasma. C-Phycocyanin and β -carotene the main antioxidants ingredient of spirulina, was found to increase HDL levels while lowering plasma levels of total cholesterol, LDL cholesterol, and triglycerides. Also, spirulina's main constituent, lowers lipid concentrations by scavenging free radicals, inhibiting lipid peroxidation, inhibiting NADPH oxidase expression, and increasing the activities of GSH peroxidase and superoxide dismutase. The lower form of nicotinamide adenine dinucleotide phosphate (NADPH) and NADH, which are cofactors in fat metabolism, may also explain the hypolipemic effects of spirulina (Serban et al. 2016). Moreover, phycocyanin can effectively inhibit pancreatic lipase and this active compound of Spirulina can bind cholesterol metabolites bile acids, so cholesterol solubility decreased and fecal excretion of cholesterol and bile acids increased (Deng and Chow, 2010).

A significant upregulation of caspase 8 and downregulation of Bcl2 were observed in CMZ-induced hypothyroidism in rats. However, a significant down regulation in caspase 8 and upregulation in Bcl-2 were observed in after treatment with GSPE and spirulina. These results were supported by Ebrahim, (2020), who reported that, the antioxidant and/or anti-inflammatory effects of dietary spirulina or GSPE reduced oxidative stress and neuronal apoptosis in diabetic rats' hippocampus. They have all been shown to play a role in either controlling the activity and the level of expression of the apoptotic initiator of caspase-8 and other genes, including caspase-3,-9, Bax, Cyt-c, TNF- α , and NF-kB genes to the lowest and upregulated the level of expression of Bcl2 gene.

5. CONCLUSION

The current study concluded that GSPE and Spirulina had therapeutic potential the treatment of hypothyroidism by improving metabolic and preventative anti-apoptotic measures against liver injury and improving thyroid hormones secretion and responsiveness.

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