Original Research Article

Grape seed proanthocyanidin alleviates toxicity, oxidative stress, injury and apoptosis involved in liver dysfunction of hyperthyroid mice

ABSTRACT

Objectives: Thyroid hormones control the basal metabolic pace of hepatocytes, and can make oxidative harm hepatic frameworks. The reason for this investigation was to investigate how hyperthyroidism-prompted liver danger, oxidative pressure and apoptosis changes could be alleviated with Grape seed proanthocyanidin separate (GSPE). This investigation assessed some biochemical, histological and immunohistochemical changes in post pubertal hyperthyroidism and its effect on liver capacity and structures. Notwithstanding the enhancing job of Grape seed proanthocyanidin remove (GSPE) supplementation was analyzed.

Materials and Methods: Fifty male Swiss albino mice were randomly divided into 5 groups (G1, Control; G2, GSPE; G3, Eltroxin-induced hyperthyroid mice; G4, Post treated hyperthyroid with GSPE; G5, Self-treated hyperthyroid mice).

Results: Our results revealed that, a significant increase in serum T3, T4, ALT, AST, ALP, liver MDA, P53 levels, injury and P53 expression in hyperthyroid mice when compared to control and GSPE. In contrast; serum albumen, liver catalase, GSH, SOD and Bcl2 were decrease in hyperthyroid mice. Treatment of hyperthyroid mice with GSPE advantages in improving the adverse effect of hyperthyroidism and moreover the histopathological and P53 expression result approves this finding.

Conclusions: GSPE can used in hyperthyroidism treatment with propylthiouracil or carbimazole or methimazole replacement therapy.

Key words: Hyperthyroidism, Grape seed proanthocyanidin, hepatic dysfunction, Oxidative stress, liver injury and PCNA.

1. INTRODUCTION

Hyperthyroidism is one of the most open endocrine issues found in medicinal practice, elevated levels in serum liver and kidney chemicals are regular going with hazard elements of hyperthyroidism [1]. Hyperthyroidism is portrayed by expanded discharge of thyroid hormones tiroiodothyronin as well as thyroxin [2,3]. Thyroxine and tri-iodothyronine are thyroid hormones that basic for typical organ improvement and metabolic capacities [4-10]. Thyroid hormones modify the basal metabolic pace of hepatocytes and along these lines control hepatic capacity [11,12]. The liver is biggest organ in the body and it assumes a significant job in our digestion where it is the principle site for lipid digestion, and the thyroid hormones have a basic impact in hepatic lipid homeostasis [13,14]. Hence it isn't astounding that expanded digestion in light of hyperthyroidism can make hepatic brokenness and oxidative harm hepatic frameworks [15]. Several plant extracts have significant antioxidant activity; one of this is a grape seeds that is rich sources for proanthocyanidins [16-19]. Proanthocyanidins are consists of many polyphenolic compounds and it have become of high importance because of their biological

properties (anti-oxidant, anti-inflammatory and anti-cancerigen) and their protective effects by reducing mitochondria damage and inhibiting cell apoptosis [20,21]. Grape seed proanthocyanidin extract (GSPE) is believed to protect against reactive oxygen species (ROS)-mediated myocardial ischemia/reperfusion injury and apoptosis [22,23]. GSPE have free radical searching properties are more noteworthy than well known cancer prevention agents, for example, nutrients C and E, and it demonstrates a better capacity than secure cells against lipid peroxidation and DNA fracture [24]. Subsequently; the present investigation was intended to explain the conceivable enhancing impacts of GSPE in improving hepatic lethality, apoptosis, oxidative pressure, damage, and apoptosis adjustments against Eltroxin instigated hyperthyroidism in male mice.

Comment [W1]: CACINOGEN

2.0 MATERIALS AND METHODS

2.1. Chemicals and drug:

ELTROXIN (Thyroxine 100 mcg; 100 Tablet) was obtained from Mercury Pharma Group Limited, Capital House, London EC4N 7BL, UK.

Grape seed proanthocyanidin (GSPE; 200mg; Pharco Pharmaceuticals Co. Alexandria, Egypt) with 95.0–96.29% Purity of Oligomeric Proanthocyanidins.

2.2. Animals

A total of 50 male Swiss albino mice (Mus musculus) 6–8 weeks old, weighing 25 ±2g, supplied from the animal house of the King Saud University, Riyadh, Saudi Arabia. Animals were provided standard mice feed and water ad libitum**.** The experimental protocol was approved by Local Ethics Committee and Animals Research, King Saud University, Riyadh, Saudi Arabia.

2.3. Experimental design and mice groups

The mice were equally divided into 5 groups with ten rats each.

G1: Control group in which mice did not received any treatment.

G2: GSPE; mice received GSPE (50mg/Kg/day) only for three weeks orally by a stomach tube [17].

G3: Hyperthyroid; mice received 100 µg/Kg Eltroxin in drinking water for 3 weeks to induce the hyperthyroid state [2].

G4: Post treated hyperthyroid; mice received 100 µg/Kg Eltroxin in drinking water for 3 weeks and then treated with GSPE for another 3 weeks (from 4^{th} week to 6^{th} week).

G5: Self-treated hyperthyroid; mice received 100 µg/Kg Eltroxin in drinking water for 3 weeks and then mice did not received any treatment for another 3 weeks (from 4^{th} week to 6^{th} week) according to Tousson et al. [13].

At the end of the experiment period, blood samples were individually collected from the eyes by retro-orbital puncture using blood capillary tubes in non-heparinized glass tubes under mild ether anesthesia. Blood samples were centrifuged to obtain serum used for detection of thyroid hormones and liver functions.

2.4. Determination of serum thyroid hormones:

Serum was used to determine the triiodothyronine (T_3) according to Thakur et al. [25]; thyroxine (T_4) according to Maes et al. [26] and thyrotropin (TSH) according to Mandel et al. [27].

2.5. Determination of serum liver enzymes

Serum was analyzed to determine aspartate transaminase (AST) and alanine transaminase (ALT) activities using commercial kit (Humann, Germany) according to the method of Schumann and Klauke [28]; serum albumin levels by using commercial kit (Diamond, Egypt) according to Doumas et al. [29]; serum alkaline phosphatase (ALP) activity in serum was detected by using commercial kit (Humann, Germany) according to Moss and Henderson [30].

2.6. Preparation of tissue homogenates

Liver from different groups were removed, weighed and stored at -20°C then 10%W/V homogenate was prepared by grand 0.3 g of tissue in 3ml of saline, liver homogenates were used to estimate the oxidative stress parameters.

2.7. Enzymatic and non-enzymatic antioxidant assays

Determination of malondialdehyde (MDA) levels in homogenate was assayed according to Mesbah et al. [31], catalase (CAT) was detected after Saggu et al. [32], superoxide dismutase (SOD) activity was detected after Misra and Fridovich [33] and reduced glutathione (GSH) content was detected after Beutler et al. [34].

2.8. Determination of P53 and Bcl2 Protein Levels

P53 and Bcl2 protein levels were determine after the method of Tousson et al. [35].

2.9. Histological preparation

The changes in the liver structure were prepared according to the method of Tousson [36].

2.10. Detection of apoptotic P53 expressions

P53 immunoreactivity were detected after the method to Tousson et al. [8,9].

2.11. Statistical Analysis

Information were communicated as mean qualities ± SE and factual examination was performed utilizing one route ANOVA to survey huge contrasts among treatment groups. The measure for factual essentialness was set at $p<0.05$ for the biochemical information. Every single measurable investigation were performed utilizing SPSS factual form 21 programming bundle (SPSS® Inc., USA).

Comment [W2]: WAS

3.0 RESULTS

3.1. Induction of hyperthyroid mice

Figures (1A-1C) showed that; the serum T_3 and T_4 levels in hyperthyroid and self-recovered hyperthyroid mice were significantly higher as compared to control; In contrast; serum TSH levels in hyperthyroid and self-recovered hyperthyroid mice were significantly lower as compared to control (Figure 1A). The treatment of hyperthyroid mice with GSPE revealed a significant decrease in T_3 and T_4 levels as compared with hyperthyroid mice (Figures 1B&1C). Also; Treatment of hyperthyroid mice with GSPE revealed a significant increase in TSH levels as compared with hyperthyroid mice group.

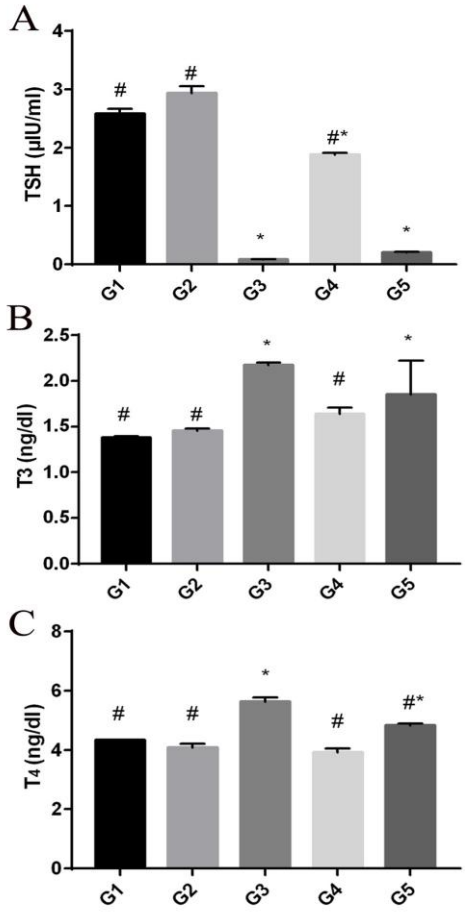


Figure 1: Changes in thyrotropin (TSH; µIU/ml), thyroxine (T4; ng/dl) and triiodothyronine (T3; ng/dl) levels in different experimental groups. Data are expressed as mean ± standard error. G1, Control; G2, GSPE; G3, Hyperthyroid; G4, post-treated hyperthyroid with GSPE; G5, Self-treated hyperthyroid. # Significantly different from G3, *Significantly different from G1.

3.2. Changes in liver functions in different groups

Table (1) showed that; a significance increase in serum ALT, AST and ALP levels in hyperthyroid mice and self-recovered hyperthyroid mice groups as compared with control and treated mice with GSPE. In contrast; significance decreases in serum albumin in hyperthyroid mice and self-recovered hyperthyroid mice as compared to control and treated mice with GSPE. On the other hand; treatment of hyperthyroid mice with GSPE revealed a significant decrease in ALT, AST and ALP levels as compared with hyperthyroid mice group. In addition to the treatment of hyperthyroid mice with GSPE revealed a significant increase in albumin levels as compared with hyperthyroid group (Table 1).

Table 1: Changes in liver functions (ALT, AST, ALP, and Alb) parameters.

	G1	G2	G3	G4	G5
ALT (U/l)	37.5$^{\#}$ ± 1.81	31.1$^{\#}$ ± 2.15	66.3* ± 4.58	40.6$^{\#}$* ± 3.19	58.4* ± 4.43
AST (U/l)	136.2$^{\#}$ ± 8.56	130.5$^{\#}$ ± 10.18	171.0* ± 10.45	134.52$^{\#}$ ± 11.22	165.1* ± 10.88
ALP (U/l)	161.9$^{\#}$ ± 11.25	147.4$^{\#}$ ± 8.69	193.1* ± 10.33	169.8$^{\#}$* ± 11.49	180.2* ± 12.05
Alb (g/dl)	4.55$^{\#}$ ± 0.216	4.78$^{\#}$ ± 0.185	3.35* ± 0.164	4.60$^{\#}$± 0.228	3.40* ± 0.157

Data are expressed as mean ± standard error. G1, Control; G2, GSPE; G3, Hyperthyroid; G4, post-treated hyperthyroid with GSPE; G5, Self-treated hyperthyroid. $^{\#}$ Significantly different from G3, *Significantly different from G1.

3.3. Lipid peroxidation and reduced glutathione content

Figure (2A) shows a significant (P<0.05) increase in the liver MDA levels in hyperthyroid and self-treated hyperthyroid mice as compared with control mice. Our data showed a significant (P<0.05) decrease in GSH levels in liver of hyperthyroid and self-treated hyperthyroid mice as compared with control mice (Figure 2B). On the other hand, hyperthyroid mice treated with GSPE showed significant depletion in liver MDA and alleviation in GSH levels as compared with hyperthyroid mice (Figures 2A&2B).

3.4. Antioxidant enzyme activities

A significantly (P< 0.05) decreased in the activities of SOD and CAT was detected in liver homogenate of hyperthyroid and self-treated hyperthyroid mice (Figures 2C&2D). Hyperthyroid mice treated with GSPE showed significant alleviation in the antioxidant enzyme activities as compared with hyperthyroid and self-treated hyperthyroid mice. Moreover, antioxidant enzyme activities were significant increase in mice treated with GSPE alone as compared to control (Figures 2C&2D).

3.5. Hyperthyroidism induced apoptosis in mice liver

Figures (3) show the concentration of P53 and Bcl2 levels in mice liver tissues. The levels of P53 were significant (P< 0.05) increase in hyperthyroid and self-treated hyperthyroid mice when compared with control (Figures 3A&3B).). While Bcl2 levels were significant (P< 0.05) depletion in hyperthyroid and self-treated hyperthyroid mice when compared with control. Treatment of hyperthyroid mice with GSPE improved this alternation of P53 and Bcl2 concentrations in liver tissues (Figure 3).

3.6. Liver histopathology

Liver sections on control and treated mice with GSPE exhibits normal histological structure of hepatocytes (Figs 4A&4B). Liver sections on hyperthyroid mice showed marked vacuolated hepatocytes, marked cellular infiltrations, inflammation, moderate degeneration with necrotic area and mild congestion in central veins and portal vein, surrounded by leucocytic infiltrations (Figures 4C&4D). Liver sections in post treated hyperthyroid mice with GSPE revealed a few vacuolated hepatocytes, moderate cellular infiltrations, and cytoplasmic vacuolations observed when compared to hyperthyroid or self-treated hyperthyroid mice (Figure 4E). In contrast; self-

treated hyperthyroid mice showed marked vacuolated hepatocytes, marked cellular infiltrations and marked degeneration with necrotic area (Figure 4F).

3.7. Changes in P53 expression

Liver sections in control group and GSPE group showed negative expression of P53 (Figure 5A&5B). Strong P53expression were detected in liver sections of hyperthyroid and self-treated hyperthyroid mice (Figures 5C&5D&5F). The intensity of P53 expression in hyperthyroid group was increased when compared with control group. Mild positive expressions for P53 were observed in liver sections in post treated hyperthyroid mice with GSPE (Figure 5E).

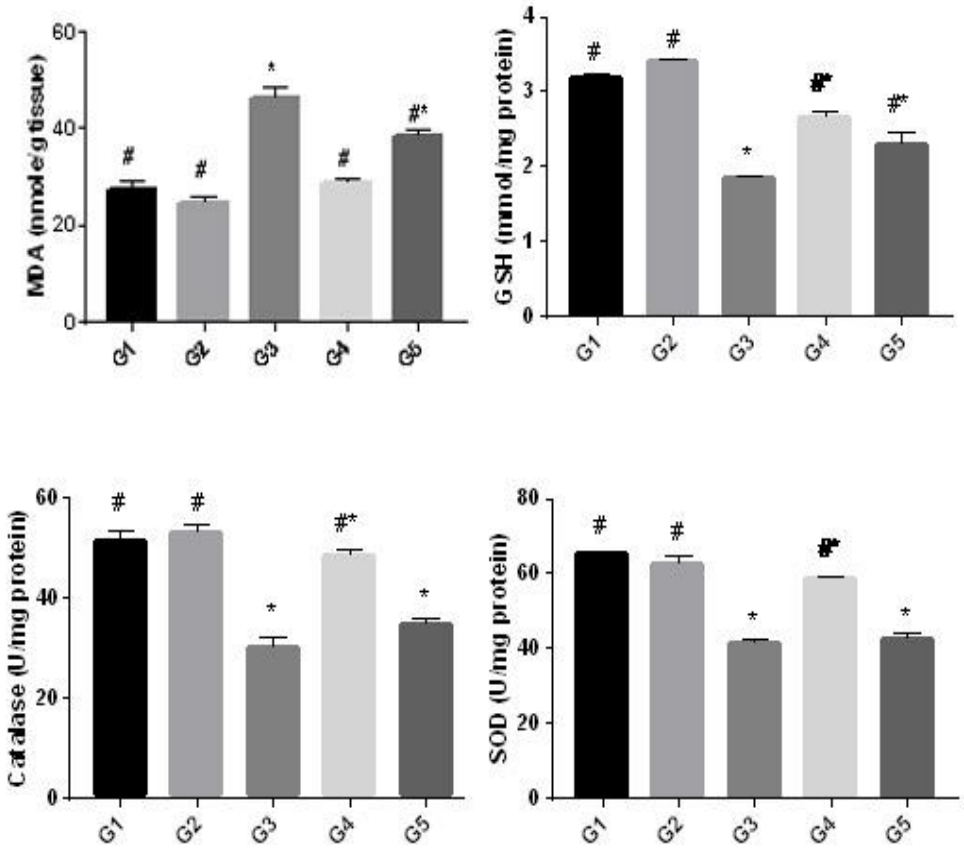


Figure 2: Changes in MDA, GSH, and catalase and SOD levels in liver tissues in different experimental groups. Data are expressed as mean ± standard error. G1, Control; G2, GSPE; G3, Hyperthyroid; G4, post-treated hyperthyroid with GSPE; G5, Self-treated hyperthyroid. # Significantly different from G3, *Significantly different from G1.

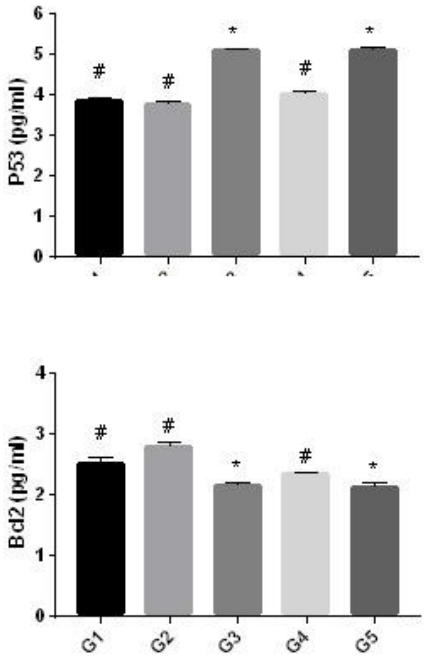


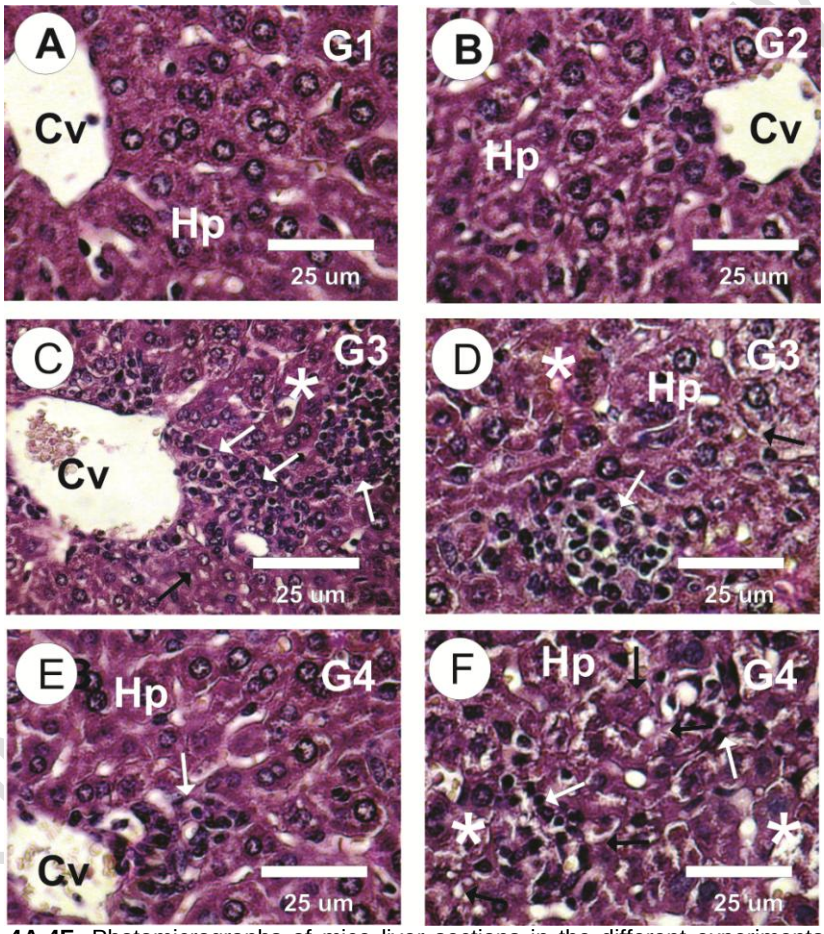
Figure 3: The changes in P53 and Bcl2 levels in mice liver tissues in all studied groups. Data are expressed as mean ± standard error. G1, Control; G2, GSPE; G3, Hyperthyroid; G4, post-treated hyperthyroid with GSPE; G5, Self-treated hyperthyroid. # Significantly different from G3, *Significantly different from G1.

4. DISCUSSION

The current study has been represented to examine the effect of a hyperthyroidism status on biochemical parameters, oxidative stress parameters, apoptosis, histological and P53 immunohistochemical alterations in liver tissues. Our results revealed elevation in serum T_3, T_4 and depression TSH in mice receiving Eltroxin indicating the hyperthyroid state. This finding is compatible with other studies that used L-thyroxin as an anti-thyroid drug for induction of hyperthyroidism [2-4]. In the current study, depletion in the level of T_3 and T_4 and elevation in TSH were detected in post treated hyperthyroid mice with GSPE when compared with hyperthyroid mice. The current outcome agrees with studies of Shibutani et al. [37]; and Beltagy et al. [2].

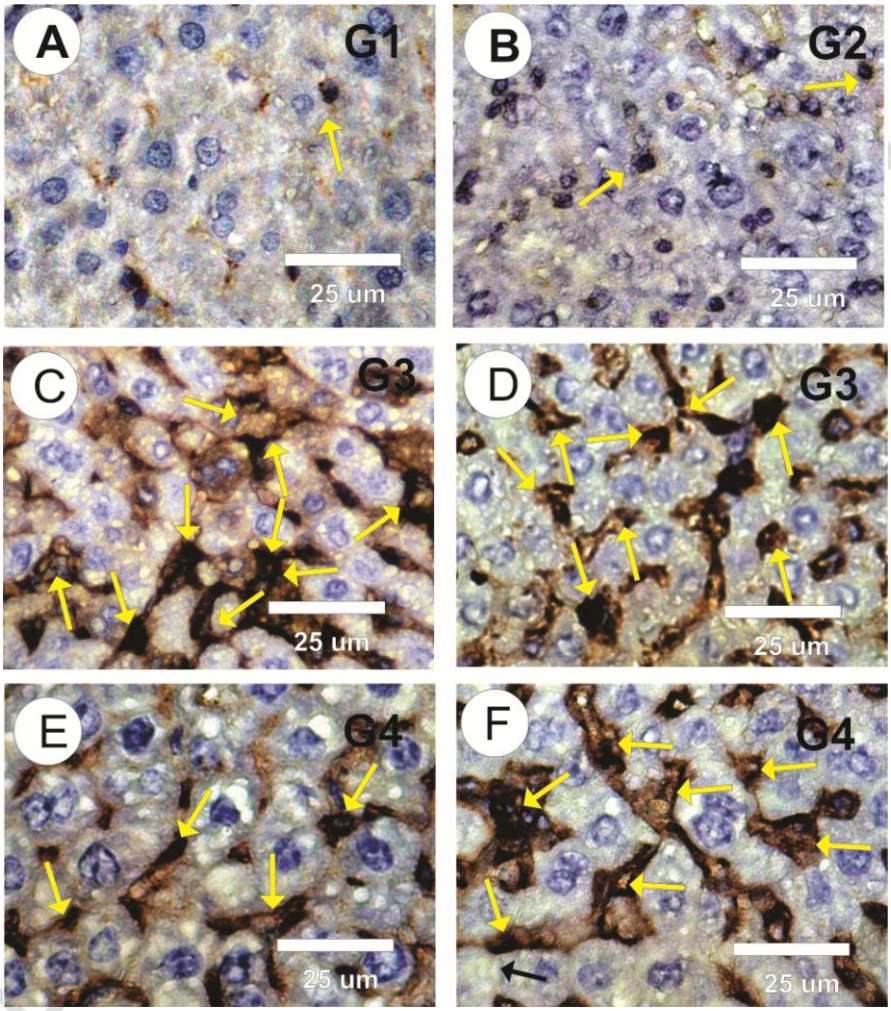
Liver assumes a significant job in thyroid hormones digestion, and correspondingly, thyroid hormones direct hepatic capacity and bilirubin digestion, in this manner as anyone might expect, disorders of either organ can possibly influence capacity of the other [12, 13]. In the current results revealed a significance increase in serum ALT, AST, ALP and significance depletion in albumen levels in hyperthyroid mice confirmed that hyperthyroidism induced dysfunction in mice

liver. Giannini et al. [38]; Hull et al. [39] fined that; a significant elevation in serum direct bilirubin, ALT, AST and ALP in hyperthyroidism. The mechanism of the elevation in serum AST, ALT and ALP activities appears to be relative hypoxia in periventricular regions of the liver [40]. Wang et al. [41] and Upadhyay *et al.* [42] who fined that; the elevation in the levels of T3 induces hepatocytes apoptosis take place through the mitochondrial dependent pathway activation. In the current results; the treatment of hyperthyroid mice with GSPE modulates liver function parameters as compared with self-treated hyperthyroidism. Current results decide with El-Sayed et al. [43] who indicated the liver protective effects of proanthocyanidin on acetaminophen. Also; Kandemir et al. [44] who studies that; the protective effects of grape seed extract against Cisplatin induced liver toxicity in rabbits.



Figures 4A-4F: Photomicrographs of mice liver sections in the different experimental groups stained with Haematoxylin & Eosin. **A&B:** Normal structure of hepatocytes (Hp) and central vein (cv) in control and GSPE groups revealed **C&D:** Marked vacuolated hepatocytes, marked necrosis (star), marked cellular infiltrations (White arrows), and moderate degeneration with necrotic area (Black arrows) in hyperthyroid mice revealed. **E**: Mild vacuolated hepatocytes

(Hp), diffuse Kupffer cells proliferation in between the some hepatocytes and moderate cellular infiltrations (White arrows) in post treated hyperthyroid mice with GSPE. **F:** Marked vacuolated hepatocytes, marked cellular infiltrations (White arrows) and marked degeneration with necrotic area (star) in self-treated hyperthyroid mice.



Figures 5A-5F: Photomicrographs of mice liver sections marked with apoptotic P53 protein. **A&B**: Negative or faint positive reactions (arrows) in hepatocyte nuclei in hepatocytes in control and GSPE. **C&D:** Strong positive reactions (arrows) in hyperthyroid. **E:** Moderate to strong positive reactions (arrows) in self-treated hyperthyroid mice. **F:** Mild positive reactions (arrows) in post treated hyperthyroid mice with GSPE.

Changes in thyroid hormone levels are known to tweak elements of numerous tissues by adjusting their cancer prevention agent resistances [45]. Hyperthyroidism is distinguished to improve ROS age and it instigates oxidative worry in liver tissue through upsetting the endogenous star oxidant/against oxidant balance [46]. In the present study, treatment of mice with Eltroxin induced hyperthyroidism that induced oxidative stress which play an important role in the damage of DNA [47]. In the current study; elevation in liver MDA and depletion in liver GSH, catalase and SOD levels in hyperthyroid mice. On the other hand, hyperthyroid mice treated with GSPE showed significant depletion in liver MDA and alleviation in GSH, catalase and SOD levels as compared with hyperthyroid and self-treated hyperthyroid mice. Asayama and Kato [48] reported that; the damage in MDA was increase in some organs in rats. Antioxidants contained in the red grape seed extract are able to inactivate superoxide anions and prevent lipid peroxidation [49]. Our results confirm GSPE as a potent antioxidant that can reduce lipid peroxidation in liver tissues. Current results agree with Davies [50] who fined that hyperthyroidism tends to increase catalase activity [51].

Comment [W3]: PLAYS

Reduced glutathione (GSH) as an oxidative stress marker showed a significant decrease in hyperthyroid rats. This decrease was improved by treatment with GSPE. Administration of GSPE exhibit an increase in antioxidant enzyme activities in hyperthyroid which might be due to its ability to reduce the accumulation of free radical generation [52]. Long et al. [52] reported that; GSPE showed an increase in GSH, catalase and SOD levels that clearly suggest their antioxidant property. GSPE has scavenging character and it can effectively inhibit liver toxicity induced by hyperthyroidism and this is may be attributed to its antioxidant properties.

Apoptosis is a urgent cell action in the conduct of mammalian cells in a wide scope of pathophysiological conditions. Apoptosis of individual cells may introduce a defensive system against neoplastic improvement in the life form by wiping out hereditarily harmed cells [41]. In the present examination; a critical increment in P53 and lessening in Bcl2 levels in hyperthyroid mice and the treatment of hyperthyroid mice with GSPE decline in P53 and increment in Bcl2 levels. Our immunohistochemical results for P53 in liver additionally affirmed these outcomes, in this way, our outcomes uncover the plausibility of the apoptosis event after Eltroxin organization. Our outcomes concur with Kumar et al. [47] who announced that; hyperthyroidism incites apoptosis in rodent liver through enactment of death receptor-interceded pathways. Likewise; Tousson et al. [8,9] accomplishment to discover backwards connection somewhere in the range of's P53 and Bcl2. Our outcomes not concur with Diebold et al [53] who neglected to uncover any connection somewhere in the range of' P53 and Bcl2.

In the current study; many signs of pathological alterations were observed in liver sections in hyperthyroid mice as marked vacuolated hepatocytes, marked necrosis, marked cellular infiltrations, and moderate degeneration with necrotic area, this outcomes perhaps were a result of oxidative pressure brought about by Eltroxin that expansion digestion and free radicals with the goal that the body's barriers of cell reinforcements can't avoid free radicals that harm liver tissue. Additionally, in our investigation on hyperthyroidism actuated in mice showed that Eltroxin hormone animates the quality answerable for customized passing in liver cells and it was seen in the histological examination a distinction in cell shading and degeneration in the cytoplasm nature just as hyperthyroidism causing ischemia in the liver [24,47,54]. In the present investigation GSPE supplementation improvement of liver harm initiated by Eltroxin, this may come back to the job of viability grape seed concentrate mixes against oxidative pressure, irritation and customized cell passing opposition [43].

In conclusion, current work confirmed that; hyperthyroidism induced liver toxicity, oxidative stress, and apoptosis also the treatment with GSPE improved these alterations in liver tissues.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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