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Protective effect of lycopene and anthocyanin against erythrosine on thyroid glands in adult male rats

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This study investigated the safety and efficacy of natural food dyes, including lycopene (LYC) and anthocyanin (ANC), as compared to synthetic colorings like erythrosine (ERY), with respect to their impact on the histological alterations within the thyroid gland and the hormonal profiles of mature male rats. Seventy-two male rats were randomly subdivided into six groups (12/group): control and ERY, LYC, and ANC rats were given 20, 5, and 200 mg/kg every other day, while the other two groups were treated with 5 and 200 mg/kg of LYC and ANC after ERY administration, respectively. The trial was continued for six weeks. In the 3rd and 6th weeks of the experiment, hormonal profile (TSH, T3, and T4) and histological examination of thyroid glands were assessed. Results indicated that ERY significantly diminished the TSH while promoting the serum T3 and T4 levels. LYC and ANC significantly ameliorated thyroid hormonal dysfunction induced by ERY. Furthermore, erythrosine caused disrupted shape, degeneration, and marked desquamation of the lining epithelium of the central follicles, as well as hemorrhage. LYC or ANC co-treatment successfully re-established and recovered near-control thyroid morphology. ERY induced a significantly higher length of the follicles and colloidal area (%) compared with other groups. LYC or ANC co-treatment significantly reduced the increase of length follicles as compared with the erythrosine group. Collectively, ERY impacted thyroid hormone homeostasis, having a considerable effect on the histological appearance of the thyroid gland in rats, while anthocyanin or lycopene resourcefully attenuated these adversative effects.

Keywords: Natural food dyes; Lycopene; Anthocyanin; Erythrosine; Thyroid gland; Thyroid stimulating hormone

INTRODUCTION

Consumer satisfaction and choices are significantly influenced by the quality of food, particularly its colors. Colors are a visual attribute related to the spectral distribution of light that interacts with matter. As a combination of sensory impressions, including sight, smell, and taste, food colors can influence the identification and acceptance of a product. Moreover, colors can also be used to warn consumers against consuming rotten or unhealthy food (Lidon and da Silva Ferreira, 2007; Egorov et al. 2020).

The potential adverse impact of food colorings on human health, particularly on young kids, is a cause for concern, as it has been linked to hyperactivity (Salvi, 2018). Moreover, studies have shown that laboratory mice exposed to synthetic food coloring or additives exhibited behavioral issues, emotional problems, and biochemical defects in their organs (Albasher et al. 2020).

Plants and plant extracts are the main traditional resources for natural food colorants. However, additional sources like animals (especially insects), algae, fungi, and bacteria (particularly cyanobacteria) are also employed to a minor extent (Gebregziabher et al. 2022). Lycopene is a carotenoid with a characteristic red color due to its conjugated polyene structure (Petyaev, 2016). This unique structure also gives lycopene its antioxidant properties (Shi and Maguer, 2000). Tomatoes and their products are the primary dietary sources of lycopene, accounting for about 80% of lycopene intake Maiani et al *(2009)* Processed tomato products, which undergo concentration via water loss, typically contain higher amounts of lycopene than fresh tomatoes (Bacanli et al. 2017). In addition to tomatoes, lycopene is present in several other fruits and vegetables, such as papaya, watermelon, carrot, pumpkin, and pink grapefruit (Böhm et al. 2003; Marković et al. 2006). Lycopene and other carotenoids have several biological properties in common, like antioxidants (Arain et al. 2018). The potential method by which carotenoids govern immunological function and cancer is associated with the involvement of carotenoids in transcriptional regulation,

apoptosis, and angiogenesis (Chew and Park, 2004).

Anthocyanins are a class of naturally occurring, pH-dependent, water-soluble food pigments ranging from purple to blue. They are members of the flavonoid family of chemicals. Anthocyanins are utilized as dietary supplements in dry-mix drinks, fruit fillings, snack bar preparation, milk products like yogurt, and confectionery preparations (like candies) because of their antioxidant and coloring characteristics (Krga and Milenkovic, 2019). Antioxidants, such as anthocyanins, are necessary for essential functions because they may scavenge free radicals and activate the body's natural defense systems (Abd El-Hack et al. 2019; Tan et al. 2022; Krga and Milenkovic, 2019). Due to their anti-oxidative and anti-inflammatory properties, anthocyanin-rich foodstuffs and anthocyanin compounds have been shown in prior human research to suppress metabolic disorders (Kolehmainen et al. 2012; Zafra-Stone et al. 2007).

Erythrosine, a red granule or powder, is commonly used in cosmetics, medicines, and food due to its water solubility (Silva et al. 2022). However, It has been linked to allergy responses and includes high quantities of bound iodine (Alagawany et al. 2022; Voss, 2002). In addition, erythrosine has been found to exhibit cytostatic and cytostatic properties in human peripheral blood cells and endotoxic and mutagenesis impacts on HepG2 cells (Chequer et al. 2012; Ishfaq et al. 2020). Long-term use of erythrosine has also been linked to adverse effects on children's thyroid function and behavioral patterns (Jennings et al. 1990). Previous research has primarily focused on examining the effects of erythrosine on the hormonal balance of the thyroid gland, while omitting a detailed analysis of its influence on the thyroid's microarchitecture. The latter signifies an indicator of thyroid impairment that is more sensitive. It is necessary to investigate the potential of natural compounds to alleviate the thyroid function imbalance induced by erythrosine. Hence, it is critical to acquire a more comprehensive comprehension of the potential fundamental mechanisms that underlie the adverse impacts of erythrosine on thyroid function, or to reassess them. The clarification of the observed alterations can be accomplished by employing comprehensive hormonal assays and histopathological assessments. By employing this methodology, the detection of efficacious protective agents, such as anthocyanin or lycopene molecules, will be facilitated.

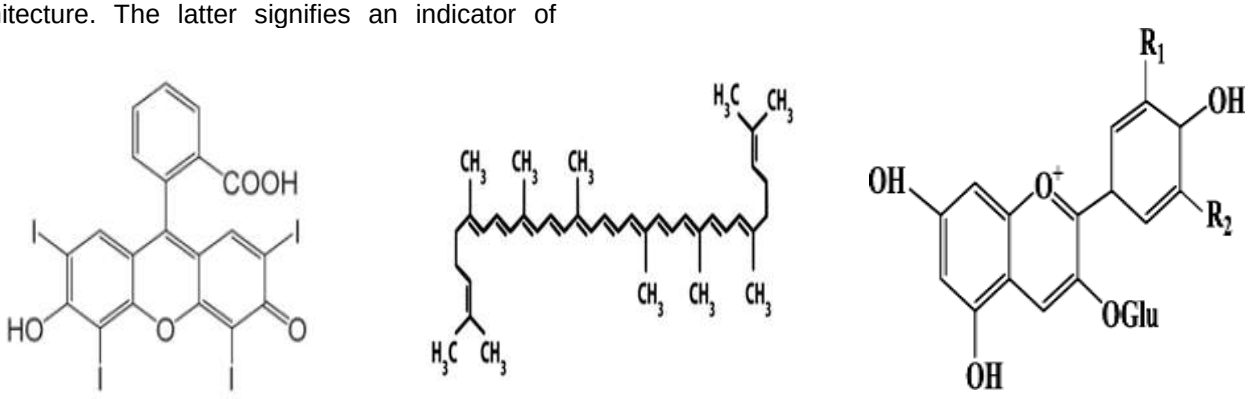
MATERIALS AND METHODS

Ethics declaration

The current research was implemented under the Animal Use in Research Committee (IACUC) at the University of Jeddah, Saudi Arabia (Ref. No. 466-21), in covenant with the ethical recommendations acceptable through the NIH (National Institutes of Health) for the Care and Usage of Laboratory Animals in scientific studies. Moreover, the ARRIVE guidelines were followed during the experiment (Du Sert et al. 2020).

Chemical reagents

Lycopene, anthocyanin, and erythrosine were perched from Sigma (St. Louis, MO, USA). The chemical structures of the previous molecules are presented in Figure 1.



A. Erythrosine B. Lycopene C. Anthocyanin

Figure 1: The chemical structures of the materials used in this experiment: A) Erythrosine, B) Lycopene, and C) Anthocyanin.

Animals

From the Department of Biology, University of Jeddah, Jeddah, Saudi Arabia, male rats (72 rats, 160 ± 0.20 g, nine weeks of age) were acquired and used in this research after one week of acclimation. Animals were kept in a well-ventilated room and placed in a stainless-steel cage with free access to water and food in a controlled room with a temperature of 22 ± 2°C, relative humidity of 50–60%, and 12 hours of light-dark cycle. For six weeks, animals were weighed and arbitrarily allotted into six groups (n = 12) that were orally given distilled water, 5 mg lycopene /kg b.w (Selim et al. 2022), 200 mg anthocyanin /kg b.w (Popović et al. 2019), 20 mg erythrosine /kg b.w (Iheanyichukwu et al. 2021), and other groups were given 20 mg of erythrosine and treated with 5 mg of either lycopene (lycopene + erythrosine) or 200 mg/kg of anthocyanin (anthocyanin + erythrosine), respectively. All treatments were performed orally via or gastric gavage between 9 a.m. and 10 a.m. every other day. Every week, the number of treatments acquired was adapted and established on the rats' body weight variability. The animal welfare, such as injury, pain, irregular behavior, discomfort, mucous membrane color, distress, morbidity, breathing patterns, and mortality, were all carefully checked through the experimentation.

Sample collection

At 3 and 6 (ending point) weeks of treatments, animals fasted for one day before sample collection. All rats were balanced and anesthetized with an intramuscular injection of xylazine (5 mg/kg b.wt) and ketamine hydrochloride (50 mg/kg b.wt). The blood samples were picked up in sterilized tubes without anticoagulants from the medial canthus. Then, blood samples in the tubes were left at room temperature for 30 min to set the serum and then the samples were centrifuged at 322 g for 20 min. The serum was carefully detached and deposited at -18°C pending hormonal investigation. Moreover, the rats were then euthanized by cervical dislocation (Aguwa et al. 2020). The rats' thyroid glands were detached throughout the autopsy, then immediately transferred and fixed with a buffered formalin solution (10% for histopathological investigation).

Serum hormonal assessments

Thyroid hormones, including TSH, T3, and T4, were assessed in serum samples using ELISA (enzyme-linked immuno sorbent assay) kits (specific for rats) according to the method of (Hwang et al. 2017) conferring the manufacturer's directions. Moreover, the Cusabio Biotech Company provided rat TSH (Catalogue number: CSB-E05115r, sensitivity: <0.3 µIU/mL detection range: 0.6– 24 µIU/mL), tri-iodothyronine (T3), (Catalogue no.: CSB-E05085r, sensitivity: 0.5 ng/mL, detection range: 0.5 – 8 ng/mL), and thyroxine (T4) (Catalogue no.: CSB-E05082r, sensitivity: 20 ng/mL, detection range: 20 -320 ng/mL) assay kits (Wuhan, China).

Histopathological examination

Thyroid glands were separated from each animal at 3 or 6 weeks of treatment, preserved in a formalin solution (10%) for fixation, then dehydrated in ascending scores of alcohol, cleared in xylene, embedded, and blocked in paraffin. A 3-micrometer thickness of each sample was stained with eosin, and hematoxylin was stained with eosin and hematoxylin following the procedure (Suvarna et al. 2018). Using a light microscope, slides were evaluated randomly by a specialized pathologist who did not know which cluster the rat fit. Moreover, morphometric analysis of thyroid glands was examined by a light microscope under magnification 40. For each variable explored in thyroid sections, ten readings per animal in each treatment were done.

Statistical analysis

The Levene and Shapiro-Wilk tests were used to check the normality and homogeneity of the obtained data. The values of these results were represented as the mean ± SEM after analyzing the data using one-way analysis of variance (ANOVA) followed by Tukey's test using SPSS software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). A P-value < 0.05 was applied to designate the significance among various groups.

RESULTS

Thyroid stimulating hormone (TSH)

The lycopene and erythrosine groups exhibited a significant decrease in TSH hormone levels at three weeks of treatment in opposition to the control. In contrast, the anthocyanin and erythrosine + anthocyanin groups showed a substantial reduction in TSH at all weeks, as shown in Table 1. However, the erythrosine group displayed higher TSH levels at six weeks than the control. Notably, the anthocyanin and erythrosine + anthocyanin groups showed a substantial decrease in TSH hormone levels relative to the erythrosine group at six weeks of experimental time.

Thyroxine (T4)

Table 2 reveals a significant reduction in T4 hormone contents in the erythrosine and erythrosine + lycopene groups at six weeks of treatment compared with the control group.

In contrast, at three weeks, the erythrosine + lycopene group exhibited an increase in T4 hormone levels compared to the control. Notably, the anthocyanin and erythrosine + anthocyanin groups showed a substantial rise in T4 hormone concentrations

throughout all weeks. Additionally, at three weeks of treatment, the erythrosine + lycopene group had a significant rise in thyroxin hormone levels compared to the erythrosine group.

Triiodothyronine (T3)

Table 3 indicates a significant enhancement in T3 hormone levels in the lycopene, anthocyanin, erythrosine + lycopene, and erythrosine + anthocyanin group at the three weeks, followed by a significant decrease in the erythrosine group at six weeks compared to the control group. Additionally, at 3 or 6 weeks, showed a substantial increase in T3 hormone contents in the lycopene, anthocyanin, erythrosine + lycopene, and erythrosine + anthocyanin groups compared to the erythrosine group.

Triiodothyronine to thyroxine ratio (T3/T4)

The data presented in Table 4 suggests a notable rise in T3/T4 hormone levels in the lycopene group at three weeks of treatment, while the anthocyanin group, erythrosine + lycopene group, and erythrosine + anthocyanin groups showed significant increases in hormone levels at six weeks relative to the control group. Additionally, significant increases in T3/T4 hormone amounts were observed in the anthocyanin group at the three weeks of treatment. Erythrosine + lycopene and the erythrosine + anthocyanin groups exhibited significant increases in hormone levels across all weeks compared to the erythrosine group

Effect of erythrosine on histological structures of the thyroid gland

At three weeks of treatment, thyroid sections from the erythrosine group revealed thyroid lobules that were considerably separated (Figure 2; A-D). A prevalence of desquamated epithelial cells was observed within the lumen of certain follicles. The follicles were predominantly lined with follicular cells characterized by vacuolated cytoplasm and dark nuclei. Certain follicles were adorned with cells that had brown nuclei that had been flattened. There is evidence that certain follicles were lined in part with numerous cellular layers. Furthermore, thyroid sections from the group that received erythrosine (Figure 2; C and D) exhibited typical histological characteristics after duration of six weeks of treatment, with the exception of a few follicles containing vacuolated cytoplasm and dark nuclei.

Effect of lycopene on histological structures of the thyroid gland

Examining thyroid gland tissue structure from the lycopene-treated group, as depicted in Figure 3 (A-D), indicated no observable changes contrasted with the control group. Following three weeks of treatment (Figure 3; A and B) and six weeks of treatment (Figure 3; C and D), the sections from the lycopene group revealed typical thyroid follicles characterized by varying sizes. These follicles were enveloped by blood capillaries and contained colloid with an acidophilic appearance, displaying peripheral vacuolations. The colloid exhibited homogeneity with diverse densities and lacked vacuolations. Notably, the follicles were lined by a singular layer of cuboidal follicular cells, each possessing spherical vesicular nuclei with prominent nucleoli (Figure 3; C and D).

Effect of anthocyanin on structural histology of the thyroid gland

The histological analysis of thyroid gland tissue structure in the anthocyanin-treated group, as presented in Figure 4 (A-D), revealed no discernible changes after three weeks of anthocyanin (Figure 4; A and B). Portions of the anthocyanin group had follicles that were both swollen and irregularly shaped. The follicular cells exhibited a range of shapes, from cuboidal to flattened. Additionally, the majority of cells had vacuolated cytoplasm and nuclei that were either spherical or flat, black, and irregular in shape. Several follicles had incomplete lining with numerous layers of cells. The interfollicular tissue exhibited congested blood arteries, and mast cells with their distinctive metachromatically stained granules were commonly seen in close proximity to these vessels in the interfollicular tissue (Figure 4; C and D).

Impact of the combination of erythrosine and lycopene on histological structures of the thyroid gland

Samples obtained from the groups that received combined erythrosine and lycopene treatment demonstrated characteristic thyroid follicles of varying diameters at the third week (Figure 5; A and B) and the sixth week (Figure 5; C and D). The follicles were enveloped by blood capillaries and were occupied with colloid, which exhibited vacuolations at the periphery and had an acidophilic appearance (Figure 5; A-D). Thyroid sections revealed a homogeneous colloid characterized by fluctuating densities and an absence of vacuolations. Furthermore, a singular stratum of cuboidal follicular cells encircled the follicles; these cells were distinguished by their conspicuous nucleoli and spherical vesicular nuclei (Figure 5; C and D).

Effect of combination of erythrosine and anthocyanin on histological structures of the thyroid gland

Sections from the groups treated with erythrosine and anthocyanin demonstrated the enlargement of specific follicles, which became irregular in shape, following a three-week treatment period (Figure 6; A-D). In addition to vacuolated cytoplasm and dark, irregular nuclei, follicular cells ranged in shape from cuboidal to flattened, with the majority of cells displaying rounded or

flat nuclei. It is worth noting that a portion of the follicles exhibited various strata of cells lining them. A congestion of blood vessels and a profusion of mast cells containing discernible metachromatically stained granules were observed in the interfollicular tissue after six weeks of treatment (Figures 6; C and D).

Effect of combination of erythrosine and anthocyanin on morphometric analyses of the thyroid gland

As clarified in Table 5, the erythrosine induced significantly higher length of the follicles and colloidal area (%) compared with other groups. However, lycopene and anthocyanin had lower percentages of connective tissue area (%) as opposed to erythrosine; these changes were non-significant. Groups lycopene + erythrosine and anthocyanin + erythrosine significantly reduced the increase of length follicles as compared with the erythrosine group. No statistical differences in epithelial thickness were observed among the experimental groups.

Table 1: Comparison of the impact of lycopene (5 mg/kg), anthocyanin (200 mg/kg), erythrosine (20 mg/kg) and their combinations on the TSH levels in adult albino rats with administration

Time	Control	Lycopene	Anthocyanin	Erythrosine	Lycopene + Erythrosine	Anthocyanin + Erythrosine
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
3rd week	0.12 ± 0.006	0.09 ± 0.004[a]	0.10 ± 0.004[a]	0.09 ± 0.002[a]	0.10 ± 0.008	0.11 ± 0.006
6th week	0.09 ± 0.004	0.08 ± 0.004	0.07 ± 0.003[a,b]	0.11 ± 0.006[a]	0.09 ± 0.008	0.07 ± 0.001[a,b]

The values of TSH were considered as µIU/mL. [a,b] Different lowercase letters within rows indicate significant differences at $P < 0.05$.

Table 2: Effect of administration of lycopene (5 mg/kg), anthocyanin (200 mg/kg), erythrosine (20 mg/kg), and a combination of lycopene or anthocyanin with erythrosine on thyroxine hormone (T4) (ng/ml) of adult albino rats

Time	Control	Lycopene	Anthocyanin	Erythrosine	Lycopene + Erythrosine	Anthocyanin + Erythrosine
	Mean ± S.E	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**
3rd week	3.10 ± 0.03	2.75 ± 0.15[b]	3.35 ± 0.14[b]	2.93 ± 0.05	3.41 ± 0.12[a,b]	3.37 ± 0.15[b]
6th week	3.58 ± 0.14	3.29 ± 0.19[b]	3.50 ± 0.11[b]	2.93 ± 0.06[a]	2.82 ± 0.25[a]	3.34 ± 0.15 [b]

The values of T4 were considered as µIU/mL .[a,b] Different lowercase letters within rows indicate significant differences at $P < 0.05$.

Table 3: Comparative impact of lycopene, anthocyanin, erythrosine, and their combinations on triiodothyronine (T3) levels in adult albino rats with administration

Time	Control	Lycopene	Anthocyanin	Erythrosine	Lycopene + Erythrosine	Anthocyanin + Erythrosine
	Mean ± S.E	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**
3rd week	2.24 ± 0.16	2.94 ± 0.10 [a,b]	2.80 ± 0.15 [a,b]	1.98 ± 0.17	3.51 ± 0.20 [a,b]	3.04 ± 0.22 [a,b]
6th week	3.35 ± 0.11	3.74 ± 0.14[b]	3.40 ± 0.13 [b]	2.98 ± 0.02 [a]	3.39 ± 0.11[b]	3.59 ± 0.10[b]

[a,b] Different lowercase letters within rows indicate significant differences at $P < 0.05$.

Table 4: The impact of administration of lycopene, anthocyanin, erythrosine, and their combinations on the T3/T4 ratio in adult albino rats with daily administration

Time	Control	Lycopene	Anthocyanin	Erythrosine	Lycopene + Erythrosine	Anthocyanin + Erythrosine
	Mean ± S.E	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**	**Mean ± S.E**
3rd week	0.70± 0.07	1.04 ± 0.08[ab]	0.84 ± 0.06[b]	0.56 ± 0.05	0.78 ± 0.06[b]	0.90 ± 0.06[b]
6th week	0.86 ± 0.04	0.89 ± 0.02	0.97 ± 0.03[a]	0.88 ± 0.04	1.10 ± 0.05[ab]	1.10 ± 0.07[a]

[a,b] Different lowercase letters within rows indicate significant differences at $P < 0.05$.

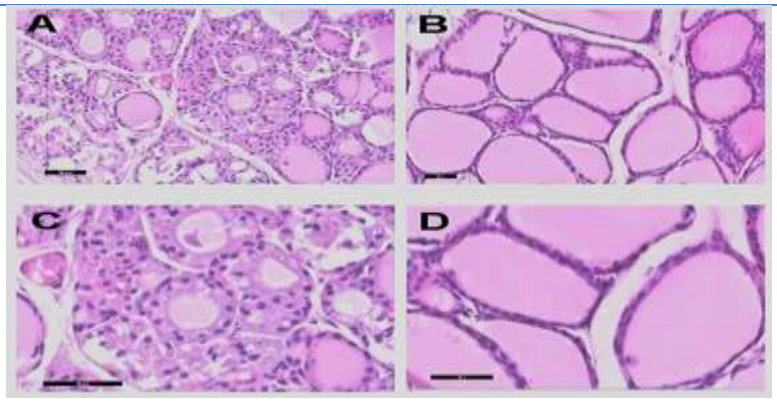


Figure 2: Effect of oral administration of erythrosine on histological structures of the thyroid gland in rats at 3rd (A, X20) (B, X40) and 6th week (C, X20) (D, X40) of treatment.

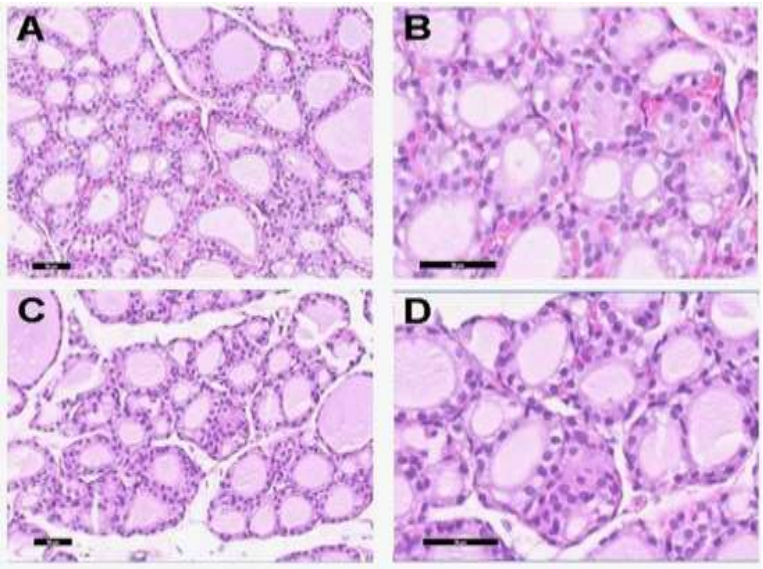


Figure 3: Effect of oral administration of lycopene on histological structures of the thyroid gland in rats at 3rd (A, X20) (B, X40) and 6th week (C, X20) (D, X40) of treatment.

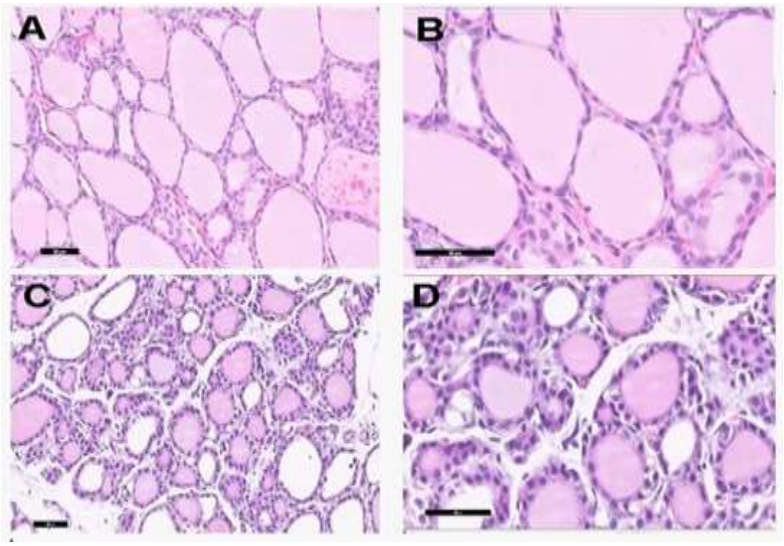


Figure 4: Effect of oral administration of anthocyanin on histological structures of the thyroid gland in rats at 3rd (A, X20) (B, X40) and 6th week (C, X20) (D, X40) of treatment.

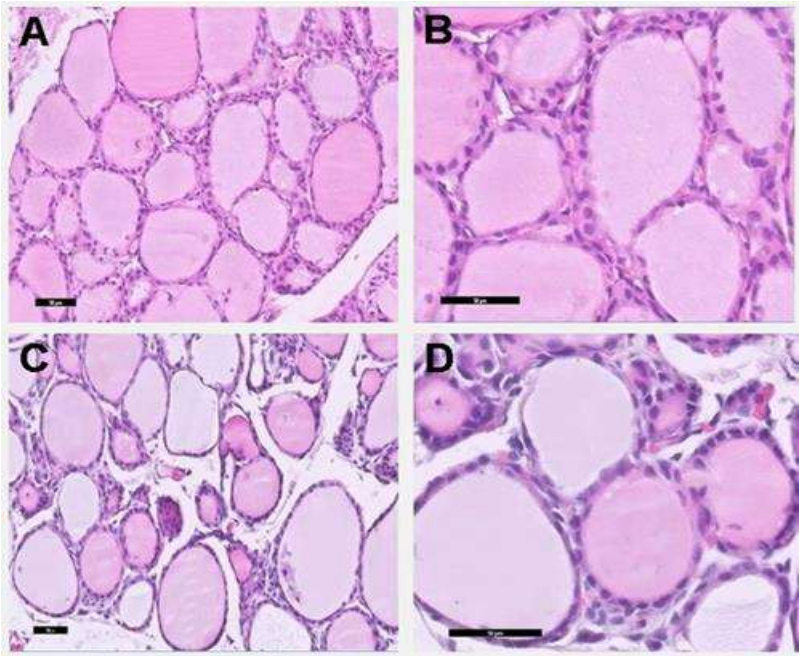


Figure 5: Effect of orally combined administration of erythrosine and lycopene administration on histological structures of the thyroid gland in rats at 3rd (A, X20) (B, X40) and 6th week (C, X20) (D, X40) of treatment.

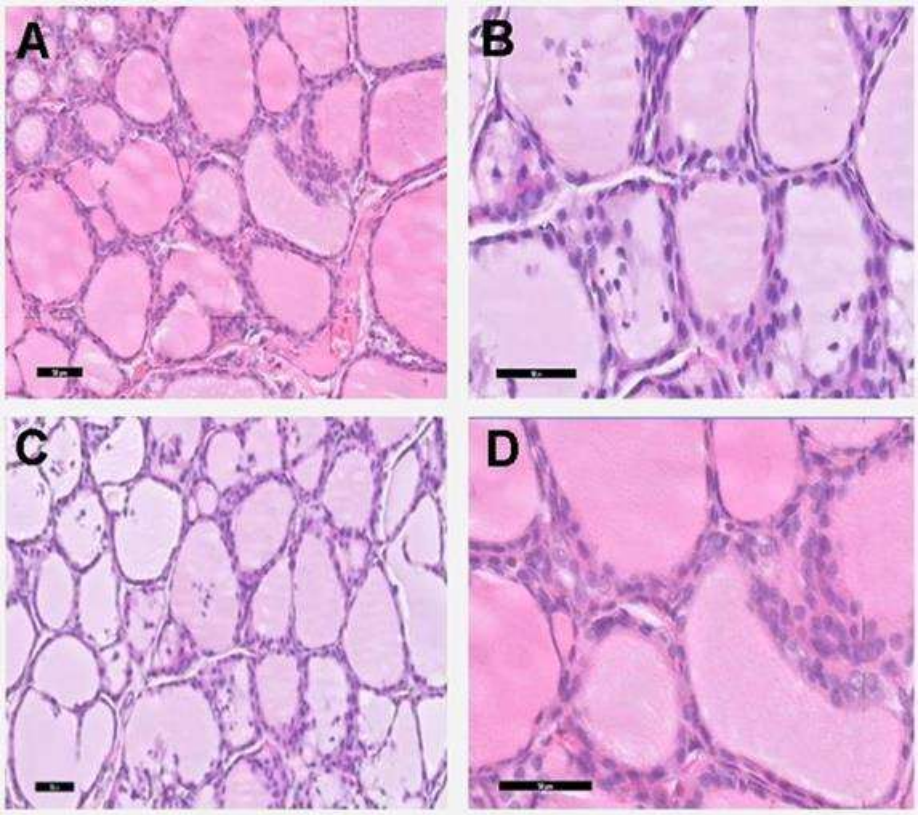


Figure 6: Effect of orally combined administration of erythrosine and anthocyanin administration on histological structures of the thyroid gland in rats at 3rd (A, X20) (B, X40) and 6th week (C, X20) (D, X40) of treatment.

Table 5: The impact of administration of lycopene, anthocyanin, erythrosine, and their combinations on the morphometric analyses of thyroid glands in adult albino rats with daily administration at 6th week

Time	Control	Lycopene	Anthocyanin	Erythrosine	Lycopene + Erythrosine	Anthocyanin + Erythrosine
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Length of the follicles (µM)	63.0 ± 2.46^{b}	62.2 ± 1.87^{b}	62.2 ± 1.78^{b}	72.9 ± 1.83^{a}	63.9 ± 1.20^{b}	63.2 ± 0. 96^{b}
Epithelial thickness (µM)	7.7 ± 0.15	7.74 ± 0.02	7.66 ± 0.03	7.76 ± 0.17	7.62 ± 0.09	7.81 ± 0.01
Colloidal area (%)	18.35 ± 0.33^{c}	19.1 ± 0.11^{c}	19.42 ± 0.29^{c}	24.6 ± 0.8^{a}	20.9 ±0.41^{b}	21.01 ± 0.26^{b}
Connective tissue area (%)	1.39 ± 0.023^{a}	1.34 ± 0.025^{a}	1.35 ± 0.06^{b}	1.11 ± 0.04^{b}	1.13 ± 0.02^{b}	1.10 ± 0.02^{b}

^{a,b,c} Different lowercase letters within rows indicate significant differences at $P < 0.05$.

DISCUSSION

Natural food dyes may be a safer alternative to synthetic dyes for the food industry, as they are less likely to impact thyroid gland function and hormone levels negatively. The findings of this study suggest that natural food dyes, such as lycopene and anthocyanin, are safer and more effective alternatives to synthetic colorings, like erythrosine, regarding thyroid gland function and hormone levels in male adult rats. According to Brent (Brent, 2012), thyroid hormones are key for operating all human organs properly. These hormones are essential for brain development, regulating metabolic rate, and other physiological processes in adults. Aguilar et al. (Aguilar F. et al. 2011) found that erythrosine exhibited a significant elevation in TSH levels and a considerable decline in T3 and T4 levels. Our data revealed that the erythrosine dye caused an increase in secretory and synthetic organelles, resulting in follicular cell hypertrophy.

These changes were consistent with mild to medium follicular cell stimulation and increased serum T4 amounts. Furthermore, rats given erythrosine had lysosomal bodies that were bigger, more erratic, and denser in electrons than controls. Also, they were fused or closely related to the limiting membrane of colloid droplets, which was a step in the thyroid hormone production mechanism. These results support the idea that erythrosine induces long-standing TSH stimulation.

The evaluation of rats' blood hormone levels in the previously mentioned study demonstrated an increase in serum TSH levels during the experimental period in the control group. The results indicated that serum TSH concentrations were significantly elevated. These observations follow the assumption that erythrosine hinders T4 and rT3 from deiodination at the 5'-position, leading to a decrease in T4 to T3 conversion and a reduction of rT3 deiodination (Braverman and DeVito, 1989).

Alshafei et al. (Alshafei et al. 2012) studied the serum concentrations of T3, T4, and TSH were determined, and histological changes were observed, including cytoplasmic vacuolation of follicular cells, pleomorphic of their blood vessels and nuclei, a rise in the number of follicular cells in multilayers, and interstitial cell hyperplasia. The group treated with erythrosine indicated a considerable drop in T3 and T4 concentrations. TSH levels showed a notable rise compared to the control group. Moreover, Thomas et al. (Thomas et al. 2015) reported that erythrosine modifies the peripheral metabolism of T4 by inhibiting the enzyme 5'-deiodinase in the kidneys and liver, which leads to decreased circulating T3 levels and an increase in reverse T3. The pituitary gland's release of TSH is increased due to the decreased circulating concentrations of T3, which stimulates thyroid follicular cells.

The current study confirmed a substantial decline in TSH concentrations and a significant rise in T3 and T4 concentrations in the lycopene-treated group vs. the control group. These hormonal results indicated a statistically noteworthy decrease in TSH contents and a significant increase in T3 and T4 levels in the lycopene-treated group relative to the control and lycopene treatments. These findings are consistent with El Dine et al. (Badr El Dine et al. 2017), suggesting that lycopene may contribute to the protection against thyroid damage and related hormones. The strong impact of lycopene on blood T4 levels may be explained by the preservation of the thyroid gland's cellular integrity and the hormones linked with it, as well as by an overall hormonal balance (Daniel et al. 2015).

In the anthocyanin group, results indicated that TSH levels were significantly dropped, but the T3 and T4 levels significantly rose. These findings are in line with Long et al. (Long et al. 2018), who indicated that mulberry anthocyanins had therapeutic potential against thyroid cancer cells by decreasing Akt, mTOR gene, and ribosomal protein S6, activating gene expression of *SW1736* and *HTh-7* gene cell death in a way that partially relies on autophagy. Anthocyanins have a structure similar to that of thyroxine hormones. They can act as antagonists by binding to the same cytoplasmic receptors as thyroxine hormones, resulting in a feedback

impact on the thyroid gland to increase production. This feedback is signaled by the hormone that releases thyrotropin (Esomonu et al. 2005; Hegedüs, 2004).

Additionally, flavonoids were found to be a strong iodothyronine deiodinase inhibitor without causing any toxicity in the intact hepatocytes and the microsomal membranes of rats. They have a high affinity and specificity for competing with T4 when competing with human T4-binding prealbumin (TBPA). However, they are ineffective inhibitors for binding 3,3',5- (T3) to the nuclear T3 receptors (Saija et al. 1990). This might be associated with erythrosine ability to induce DNA instability in thyroid glands by increasing the expression of the DNA mismatch repair system (TP73, MLH1, and MSH3) (Chequer et al. 2017; Raza et al. 2020). The previous genes are critical for maintaining genome stability (Jiricny, 2006). These verdicts were in line with earlier reports regarding the protective effects of lycopene against chemical–disturbing thyroid function (Abdul-Hamid and Salah, 2013; Ibrahim et al. 2021). Moreover, lycopene effectively re-established the healthy assembly and homeostasis of the thyroid gland after exposure to pesticides (Abdul-Hamid and Salah, 2013) or food colorants Aroclor 1254 (Ibrahim et al. 2021) with endocrine-disrupting action in rats. This protective effect of lycopene might be attributed to its anti-oxidative ability and anti-mutagenic effect.

The results indicated that anthocyanin administration increased serum prolactin in male rabbits, consistent with Okasha et al. (Okasha et al. 2008) (Colao and Lombardi, 1998). Flavonoids and phenolics are similar to T4 hormones and may compete with them for cytoplasmic receptors, acting as antagonists. This feedback effect may increase the production of thyroxine by the thyroid gland, which is signaled by the thyrotropin-triggering hormone (Esomonu et al. 2005; Hegedüs, 2004; Roberts and Ladenson, 2004). Moreover, this signal may boost prolactin production (Esomonu et al. 2005). Regarding anthocyanin, several trials strongly proposed the positive effects of anthocyanin in attenuating oxidative stress (Tian et al. 2022) and supporting the anti-oxidative status(Raza et al. 2021), where it is reflected as one of the most effective phytochemical antioxidants (Chen et al. 2022), widely used for sustaining the integrity of the tissue such thyroid follicular. However, no clear report has been found conferring its protective effect against ERY-induced thyroid dysfunction. Moreover, anthocyanin could mitigate lung destruction in rats by decreasing DNA damage and oxidative stress (Amararathna et al. 2020). Moreover, anthocyanin can significantly repress the proliferation and encourage apoptosis via constraining Sirt1/survivin and Akt/mTOR paths alongside breast tumor cells (Layosa et al. 2021).

It is well-known that changes in the histological and morphometric variables in thyroid glands might be linked with alteration of serum thyroid hormone levels, thus affecting the thyroid function. In this study, we observed that the erythrosine induced significantly higher length of the follicles and colloidal area (%) compared with other groups. Groups lycopene + erythrosine and anthocyanin+ erythrosine significantly reduced the increase of length follicles as compared with the erythrosine group. Several studies have revealed that the intracytoplasmic colloid vesicles caused a decrease in thyroid activity.

Moreover, this potential effect of anthocyanin was performed via activating the antioxidant signaling path controlled by Nrf2 (nuclear factor erythroid 2) in tumor cells, showing a significant part in antioxidant hindrance (Roy and Rhim, 2021) and greater hydroxylation on the B ring of polyphenols (Raza et al. 2021). Inflammation of the erythrosine in the histopathological changes in thyroid glands has also been performed in this research. The association between inflammation and Ferro ptosis needs further clarification to assess more changes in the structural stability of thyroid glands in response to natural molecules used in this study. Moreover, to gain more insight into the potential effects of erythrosine in the thyroid or other glands in the body, transcriptomics, metabolomics or proteomics are urgently required.

CONCLUSIONS

Natural food dyes like lycopene and anthocyanin appear to be a safer and more effective alternative to synthetic dyes like erythrosine regarding thyroid gland function and hormone levels in male adult rats. Studies have shown that erythrosine can induce long-standing TSH stimulation and cause hypertrophy for the follicular cells with increased synthetic and secretory organelles. However, in rats, lycopene and anthocyanin administration have significantly decreased TSH levels and increased T3 and T4 levels. Furthermore, anthocyanins are effective, non-toxic inhibitors of iodothyronine deiodinase and may act as antagonists, resulting in a feedback impact on the thyroid gland to trigger the release of more thyroxin. These findings suggest that natural food dyes may be safer than artificial dyes for the food industry.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/10.3390/antiox12081524/s1,

Author contributions

Conceptualization, methodology, software, F.A.A. and F.A.; validation, formal analysis, F.A., and H.A.N.; investigation, resources, F.A.A. and F.A.; data curation, writing—original draft preparation, F.A.A. and F.A.; writing—review and editing, H.A.N. and F.A.A.; funding acquisition, F.A.A. and H.A.N. All authors read the present manuscript and they accepted this version.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data of this research are available upon request.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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