



Physiological Study of Broccoli Extract Effects on some Blood Parameters, Thyroid Hormones and Histological Changes Male Albino Mice

Montaha Khalaf Al-Kinany^{1*}  , Ayyad W. Al-Shahwany²  

^{1,2}Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

*Corresponding Author

Received: 26/December/2025

Accepted: 11/June/2026

Published: 20/October/2026

<https://doi.org/10.30526/39.4.4363>



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Abstract

The present study aimed to investigate the effects of Broccoli extract on some blood indices, coagulation parameters, thyroid hormone levels, and the serum lipid profile in male mice. Adult male mice (n=20) were randomly divided into the control group (n=10) received a standard laboratory diet only and the treated group (n=10), which were dosed with broccoli extract (200 mg/kg body weight) once daily for 25 consecutive days. The hematological profile showed no modifications on white blood cell count (WBC), hemoglobin level (Hb), hematocrit and platelet count, showing that the extract didn't alter major hematological parameters. However, there is no significant change in partial thrombin time (PT) and slight but non-significant increase in activated partial thromboplastin time (aPTT) indicating a possible mild effect of broccoli extract on intrinsic coagulation pathway. Thyroid hormones assessment revealed a notable reduction in TSH (0.090 ± 0.01 vs 1.033 ± 0.03 μ IU/ml), significant increase in T4 level (14.13 ± 0.55 vs 9.20 ± 0.10 μ g/dl) while T3 show a nonsignificant elevation (2.866 ± 0.29 vs 1.733 ± 0.03 ng/ml) suggesting improve the thyroid function. Serum levels of total cholesterol remained unchanged, suggesting no immediate effect on lipids. Histopathologically, liver and kidney tissues showed vascular congestion, necrosis, and inflammatory infiltration, while the spleen showed moderate shrinkage, suggesting an early stress response and mild tissue toxicity. In general, it can be concluded that broccoli extract has mild, non-significant anticoagulant effect and thyroid-promoting activities, whereas more studies are required to verify long-term safety and clinical relevance.

Keywords: Broccoli, *Brassica Oleracea*, Glucosinolates, Hematological parameters, Thyroid hormones, Mice.

1. Introduction

The family Cruciferae is one of the largest families in plant kingdom which is famous for medicinal plants and nutritive value. It is comprised of 338 genera and 3,350 species, which are widely distributed¹. Broccoli known source of nutrients and health-enhanced compounds, such as vitamins, dietary fibers, minerals and an accumulation of phytochemicals (mainly glucosinolates, flavonoids and phenolic compounds) with antioxidant and anti-inflammation activity². Glucosinolates (GSL) sulfur-containing secondary metabolites found in cruciferous vegetables including broccoli. These biologically active compounds are regarded the hallmarks of broccoli due to their health advantages. GSLs compound upon consumption, will undergo enzymatic hydrolysis by the myrosinase enzyme (which found in plant and gut bacteria), yield nitriles and allyl isothiocyanates, which are particularly known for their potent anticancer and detoxifying effects³. Recent studies have been shown that the bioactive compounds of broccoli especially GLS affect coagulation pathways by prolonging prothrombin time (PT) and activated partial thromboplastin time (aPTT) and reducing platelet aggregation and thrombus formation⁴. Thyroid hormones (T₄ and T₃) regulate metabolism, growth, development, and

sustain physiological systems. These hormones are controlled by the hypothalamus-pituitary-thyroid. The hypothalamus releases TRH, which causes the pituitary to release TSH, regulating T_4 and T_3 levels. Hypothyroidism and hyperthyroidism interrupt this homeostasis, affecting metabolism, cardiovascular function, thermoregulation, mood, and cognition ⁵. Recent studies have demonstrated that many plant extracts and phytochemicals, including flavonoids, polyphenols, glucosinolates, and others, may affect thyroid hormone level or thyroid gland function ^{6,7}. Reports indicate that the glucosinolates (GLSs) and isothiocyanates compounds have antioxidant and cytoprotective role. These phytochemicals regulate thyroid hormone metabolism and prevent chemically caused hypothyroidism by maintaining T_3 , T_4 , and TSH levels ⁸. Cholesterol is an important lipid molecule that helps keep cell membranes strong and makes steroid hormones. But having high levels of total cholesterol in the blood is a major risk factor for heart disease. Numerous studies have examined plant-derived extracts for their capacity to influence lipid metabolism, with certain reports demonstrating notable hypocholesterolemic effects, while others reveal negligible impact, depending on the extract type and bioactive chemicals ^{9,10}.

Despite the well-documented therapeutic efficiency of broccoli extract and its bioactive compounds, including their effect on coagulation and hormones have been documented, as mentioned in the studies above, limited in vivo studies have comprehensively studying the simultaneous effects on blood parameters, coagulation, hormones, and physiological changes under normal condition and healthy mice model. Therefore, it was hypothesized that broccoli extract, which is rich in active compounds such as glucosinolates, affects blood clotting factors and the level of thyroid hormones, and can cause histological changes at the tested dose.

Accordingly, this study aimed to assess the impact of broccoli extract on hematological indices, coagulation status, thyroid hormone regulation, and serum cholesterol levels in healthy mice.

2. Materials and Methods

2.1. Collection of plant sample

Broccoli was purchased from a local vegetable market in Baghdad, Iraq. The species (*Brassica oleracea* var. *italica*) was identified and classified in BUH (College of Science Herbarium, Biology Department, University of Baghdad, Baghdad, Iraq). The leaves of broccoli were thoroughly washed with tap water. Afterwards, leaves were dried in an oven for 24hr at 40-45°C until dry. Grinding the plant part and storing them at 4°C until use.

2.2.Extraction of plant sample

A powdered sample of Broccoli leaves(50g) was extracted with 500 mL of 80% methanol and left at ambient temperature for 24 h. Extract was centrifuged at 1780 rpm for 20 min at 4 °C, and the supernatant was filtered through 0.45 µm PVDF membrane filters. Filtrates were stored at low temperature until further analysis ¹¹.

2.3.Quantitative Determination of Bioactive Compounds

Broccoli extract is recognized as a rich source of health-benefit compounds, including glucosinolates, phenolic compounds, flavonoids, alkaloids, and other phytochemicals ¹². In this study, quantitative assessment of the major bioactive constituents in broccoli extracts was conducted using validated analytical protocols with minor methodological adjustments. The employed assays and their detection principles are summarized in **Table 1**.

Table 1. Analytical methods for quantitative determination of bioactive compounds in broccoli.

Bioactive compound	Method principle
Total Phenolic Content (TPC)	Folin–Ciocalteu colorimetric method; absorbance at 760 nm; expressed as mg GAE/g dw ¹³
Total Flavonoid Content (TFC)	Aluminum chloride colorimetric method; absorbance at 510 nm; expressed as mg QE/g dw ¹⁴
Total Alkaloid Content (TAC)	Bromocresol green (BCG) complex formation; absorbance at 470 nm; expressed as mg AE/g dw ¹⁵
Total Glycoside Content (TGC)	Baljet's reagent colorimetric method; absorbance at 495 nm; expressed as mg SE/g dw ¹⁶
Total Saponin Content (TSC)	Gravimetric method with aqueous ethanol, diethyl ether, and n-butanol extraction; expressed as mg/g dw ¹⁷
Total Tannin Content (TTC)	Protein precipitation with gelatin; absorbance at 540 nm; expressed as mg TAE/g dw ¹⁸
Total Glucosinolates	Sodium tetrachloropalladate colorimetric method; absorbance at 425 nm; calculated using $y = 1.40 + 118.86 \times A_{425}$ ($\mu\text{mol/g}$) ¹⁹

2.4. Experimental Animal and Design:

Twenty male albino mice (*Mus musculus albino*), aged 6 weeks and weighing 25–30 g, were obtained from the Biotechnology Research Center, Al-Nahrain University. Animals were maintained under standard laboratory conditions (controlled room temperature, adequate ventilation, and appropriate humidity), with free availability of food and water. Mice were randomly divided into two groups (n = 10 each):

- Control group: received a standard laboratory diet only.
- Treatment group: administered broccoli extracts orally at a dose of (200 mg/kg body weight) using an oral feeding syringe (once daily).

Each group was housed in a separate cage to avoid cross-interference. The experimental period continued for 25 days. One dose of broccoli extract of biological relevance (200mg/kg of body weight) was chosen based on previous studies²⁰, since the objective of the research was to evaluate the basic physiological action of the plant extract, rather than comprehensive determination of dose-response profile. Besides that, the efficiency of one effective dose reduced the number of animals to be used, which is ethically appropriate to the 3R principle of (Reduction, Refinement, Replacement).

2.5. Collection of blood sample and organ

Blood samples were collected from the animals under light anesthesia on the final day of the experimental period. Approximately 1 mL of blood was collected using a medical syringe. All the samples meant for hematological examination were then dispensed into EDTA-containing tubes for quick prevention of coagulation and kept slant at 4°C in the refrigerator for analysis. For serum separation, the blood was centrifuged at 3000 rpm for 15 min to make sure that serum was completely separated from RBC and allowed to clot in serum gel separator tubes. After separation, the serum was put in separate, sterilized, labeled Eppendorf tubes and stored at –20 °C until used for further analysis²¹.

After the treatment period, all mice were euthanized. The liver, kidneys and spleen were removed, washed with physiological saline solution and fixed immediately in 10% neutral buffered formaldehyde for histopathological evaluation.

2.6. Hematological and Coagulation Analysis

Hematological parameters were analyzed with blood specimens collected in EDTA and anticoagulated. A complete blood count (CBC) study was performed using an automated hematology analyzer²², which provided quantitative values of white blood cell count (WBC), packed cell volume (PCV), platelet count, and hemoglobin concentration (Hb). The obtained results were used to investigate possible hematological changes after broccoli extract administration.

Measures of PT and aPTT were performed on a Sigma Amelung KC4Δ micro-coagulation analyzer (alternatively a Diagnostica Stago Start Hemostasis Analyzer) using RecombiPlasTin 2G thromboplastin (Instrumentation Laboratory) of PT and aPTT-SP using 0.025 M CaCl₂ of aPTT. Plasma (50 μL) was tested in two folds. PT was started with 100 μL thromboplastin after a 1-min incubation, and aPTT was started with the addition of 50 μL APTT reagent at 1 min and incubation was stopped by the addition of 50 μL CaCl₂ on PT. QC was done on pooled mouse plasma and commercial normal/abnormal control pools daily; imidazole buffer and factor-deficient plasmas were used to generate factor activity curves by 1:10 serial dilution of pooled plasma²³.

2.7. Biochemical analysis

2.7.1. Measurement of Thyroid Hormones:

Biochemical analyses were performed by means of established methods to measure serum concentrations of TSH and T₃ as previously described²⁴, and T₄ as reported in²⁵. Hormones were assayed according to standard spectrophotometer or immunoassay procedures.

2.7.2. Determination of Serum Cholesterol (CHO):

The total serum cholesterol was determined using the method described in²⁶. The procedure is based on enzymatic hydrolysis of the cholesterol esters and subsequent oxidation reactions through red-colored complexes with spectrophotometrically measurements.

2.8. Histological study

The organ (spleen, kidney, and liver) was obtained and fixed by immersion in 10% buffered formalin. After fixation, the organs were then dissected in a systematic pattern and processed for paraffin embedding. The 3μm sections were used for hematoxylin-eosin (H & E) staining for light microscopic description²⁷.

2.9. Statistical analysis

Statistical analyses were conducted using SPSS software (version 26, IBM, 2019). Results are expressed as Mean ± SE Statistical analysis. The difference of means between treatments and control was tested using Student's t-test to confirm the significance of the difference. Before analysis, data were evaluated for normality distribution. P-values of ≤ 0.05 were regarded as significant, and those $P > 0.05$ were considered not significant (NS)²⁸.

3. Results

3.1. Determination of Bioactive Compounds

Quantitative analysis of the broccoli extract (**Table1**) revealed high amounts of total phenolics and flavonoids, explaining its powerful antioxidant capacity (**Figure 1**). Glucosinolates were also more abundant, Tannins and alkaloids were at moderate levels, while Glycosides and Saponins had the lowest concentration.

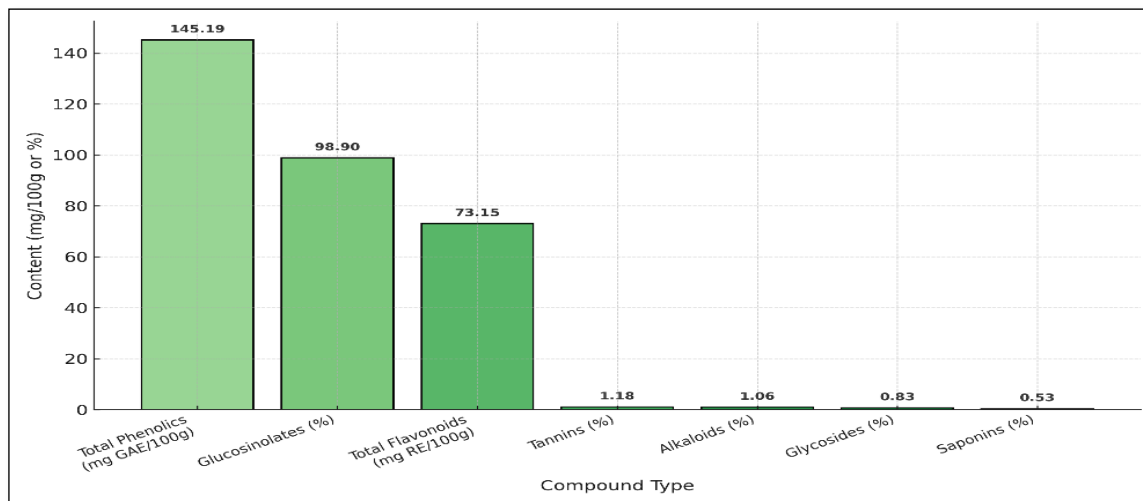


Figure 1: Distribution of bioactive compounds in broccoli extract.

3.2. Hematological parameters and coagulation time

Analysis of the blood profile **Table (2)** shows no significant differences between the control and treated groups in WBC count, hemoglobin (Hb), and packed cell volume (PCV); these results may indicate that broccoli extract did not produce obvious hematological disturbance. There were slight but non-significant increases in platelet numbers of the treated group. There is no significant change in PT and there slight but non-significant increase in aPTT after treatment vs controls.

Table 2. Effect of Broccoli Extract on Hematological and Coagulation Parameters in Mice

Group	Means $\pm$ SE					
	WBC ($10^3/\mu\text{L}$)	Hb (g/dl)	PCV (%)	PLT ($10^6/\mu\text{L}$)	P.T / Sec.	aPTT /Sec.
Control	9.27 $\pm$ 2.13	9.10 $\pm$ 0.91	31.67 $\pm$ 2.96	1.314 $\pm$ 0.14	13.33 $\pm$ 1.85	19.00 $\pm$ 1.52
Treatments	8.63 $\pm$ 0.81	8.93 $\pm$ 1.48	31.00 $\pm$ 5.03	1.829 $\pm$ 0.51	12.67 $\pm$ 1.33	23.33 $\pm$ 1.86
T-test	1.336 NS	1.910 NS	4.216 NS	1.465 NS	3.344 NS	3.892 NS
P-value	0.7950	0.9283	0.9146	0.3841	0.7850	0.1498

* ($P \leq 0.05$), NS: Non-Significant.

3.3. Biochemical analysis

3.3.1. Cholesterol level

Blood serum analysis shows no significant variance in cholesterol level between the control and treatment groups, as shown in **Table 3**.

Table 3. The impact of broccoli extract on serum cholesterol levels in mice.

Group	Mean $\pm$ SE of Cholesterol (mg/dl)
Control	145.00 $\pm$ 3.22
Treatments	154.67 $\pm$ 6.96
T-test	1.261 NS
P-value	0.2579

NS: Non-Significant.

3.3.2. Thyroid hormones

Thyroid hormone analysis revealed a notable reduction in TSH levels and substantial increases in T4 concentrations in the treated group relative to the control, which was statistically significant ($p \leq 0.01$) while there is no significant elevation in T3, **Table 4**.

Table 4. Influence of Broccoli Extract on Serum TSH, T3, and T4 Levels in Mice

Group	Means $\pm$ SE		
	TSH ($\mu\text{IU/ml}$)	T3 (ng/ml)	T4 ($\mu\text{g/dl}$)
Control	1.033 $\pm$ 0.03	1.733 $\pm$ 0.03	9.20 $\pm$ 0.10
Treatment	0.090 $\pm$ 0.01	2.866 $\pm$ 0.29	14.13 $\pm$ 0.55
T-test	0.0925 **	0.8278 NS	1.565 *
P-value	0.0001	0.060	0.011

** ($P \leq 0.01$).

3.4. Histopathological effect:

3.4.1. Control Group Versus Treated Group (liver, kidney, spleen)

• Liver (control versus treated)

Histological evaluation of the control group revealed normal tissue architecture in all examined organs. The liver (control) exhibited a typical arrangement of hepatic cords radiating from a centrally located vein, with intact hepatocytes and normal sinusoidal spaces (**Figures 2 and 3**). Marked pathological alterations were observed in the treated group. While liver sections in treated group revealed severe vascular congestion with dilation of the central and portal veins (**Figure 4**), along with pericentral mononuclear leukocyte infiltration, hepatocellular necrosis, and hemosiderin-laden macrophages (**Figure 5**). The portal triad showed intense periportal mononuclear leukocyte aggregation with vascular congestion (**Figures 6 and 7**).

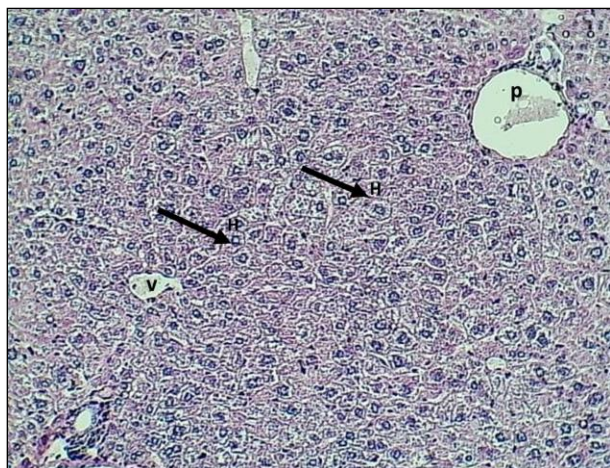


Figure 2. Section of liver (Control) revealed normal central vein (V), portal vein (p), normal arrangement of hepatic cords (H). H&E, 10x.

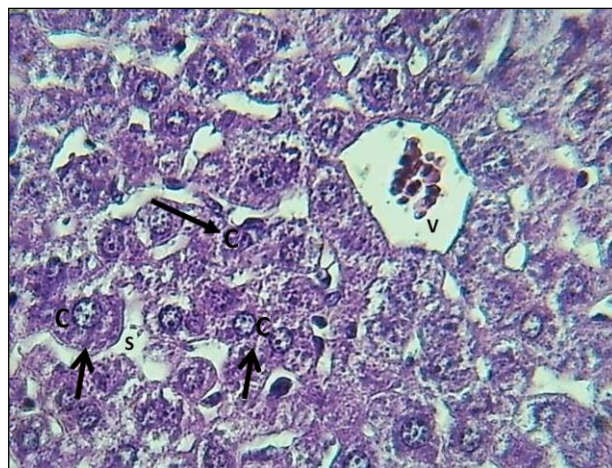


Figure 3. Section of liver (Control) shows normal appearance of the central vein (V), normal appearance of hepatocyte (C) & sinusoids (S). H&E, 40x.

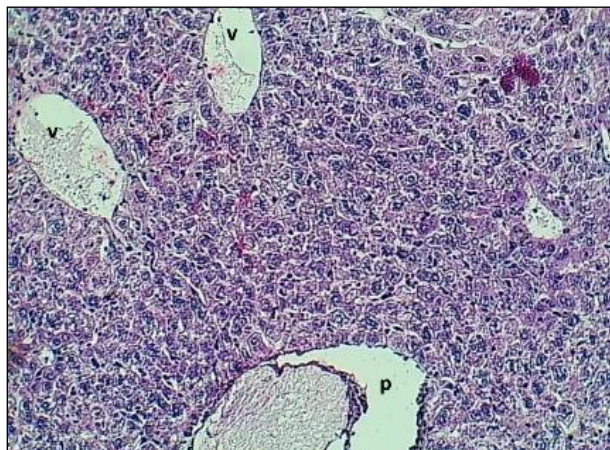


Figure 4. Section of liver (treated) shows marked dilation with central (V) and portal (p) veins. H&E stain, 10x.

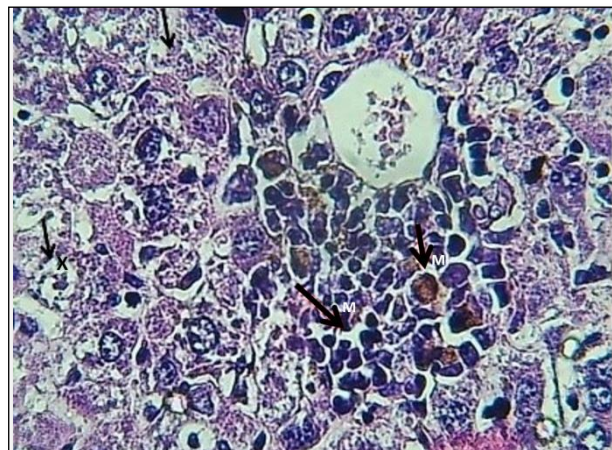


Figure 5. Section of liver (treated) showed peri central aggregations of MNLs with marked hemosiderin laden macrophages (M) & necrosis of hepatocyte (X). H&E stain, 40x.

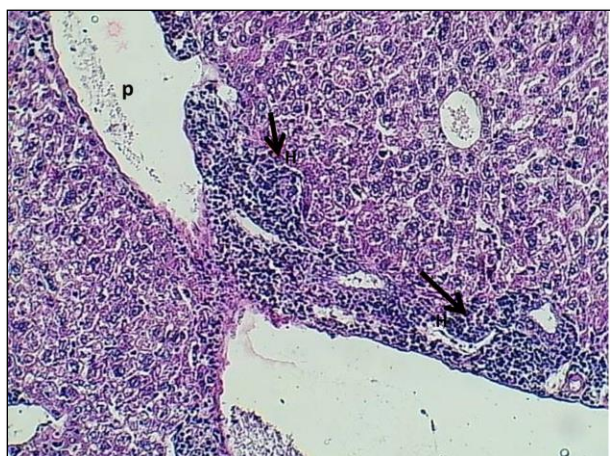


Figure 6. Section of liver portal triad (treated) showed peri portal aggregations of MNLs (H) with congestion of portal vein (P). H&E stain, 10x.

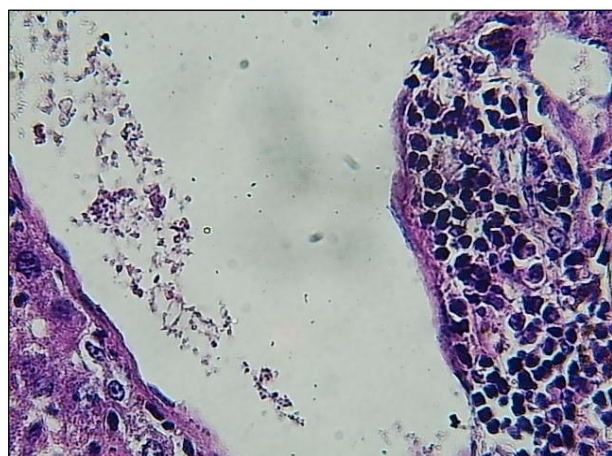


Figure 7. Section of liver portal triad (treated) showed peri portal aggregations of MNLs with congestion of portal vein. H&E stain, 40x

- Kidney (control versus treated)

Renal sections (control) showed preserved morphology of the cortex and medulla, with intact renal tubules and glomeruli free from degenerative or inflammatory changes (**Figures 8 and 9**). While renal tissues in treated group exhibited severe interstitial nephritis characterized by extensive tubular epithelial necrosis, dense mononuclear leukocyte infiltration, focal tissue depletion, and glomerular alterations (**Figures 10 and 11**). Additional sections showed intraluminal cellular desquamation, Bowman's capsule metaplasia, and marked interstitial hemorrhage (**Figures 12 and 13**).

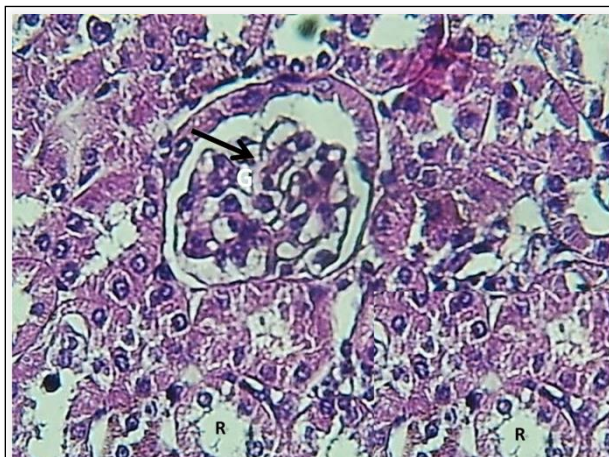


Figure 8: Section of renal cortex (control) shows normal lining cells of renal tubules (R), with normal architecture of glomerulus (G). H&E stain, 40x.

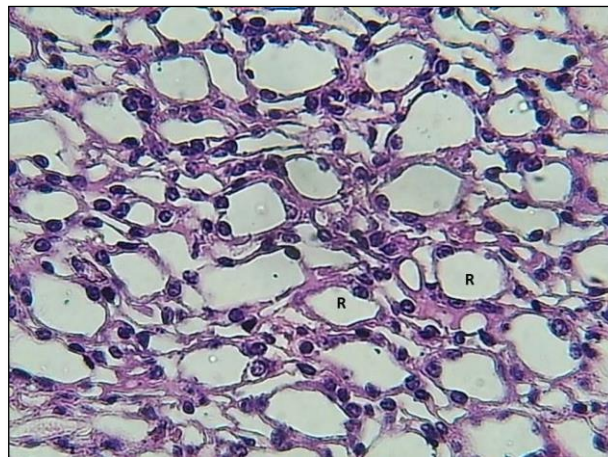


Figure 9: Section of renal medulla (control) shows normal lining cells of renal tubules (R). H&E stain, 40x.

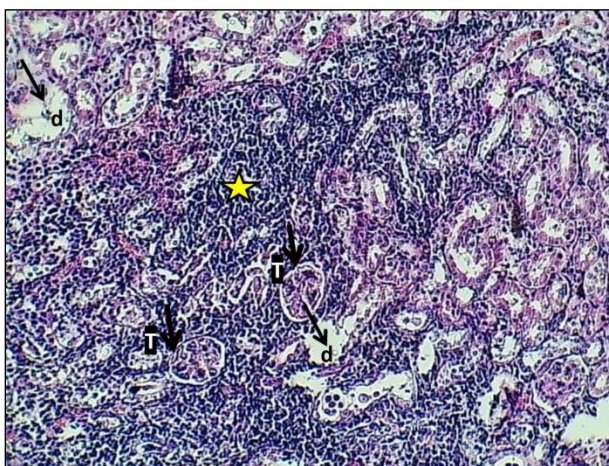


Figure 10: section of renal cortex (treated) shows severe interstitial nephritis that revealed severe necrosis of lining cells of renal tubules with infiltration of MNLs (asterisks), tissue depletion (d) & glomerular congestion (T). H&E stain, 40x

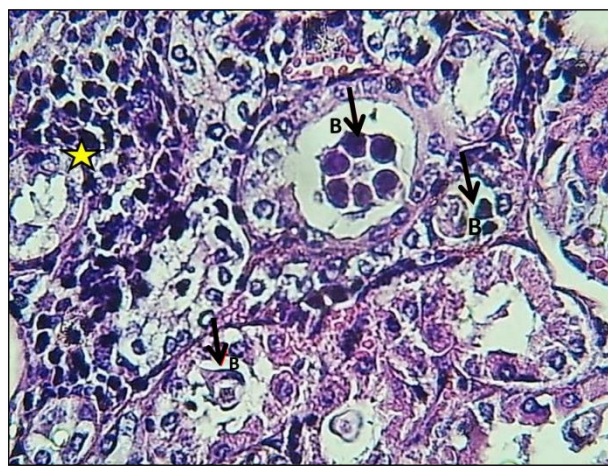


Figure 11: section of renal cortex (treated) shows severe necrosis of lining cells of renal tubules with infiltration of MNLs (asterisks) & intra luminal cellular desquamation (B). H&E stain, 40x

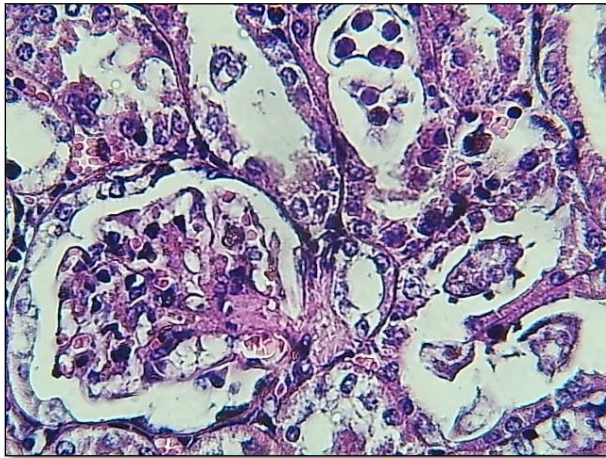


Figure 12: section of renal cortex (treated) shows severe necrosis of lining cells of renal tubules with intra luminal cellular desquamation marked metaplasia of glomerular bowman capsule. H&E stain, 40x.

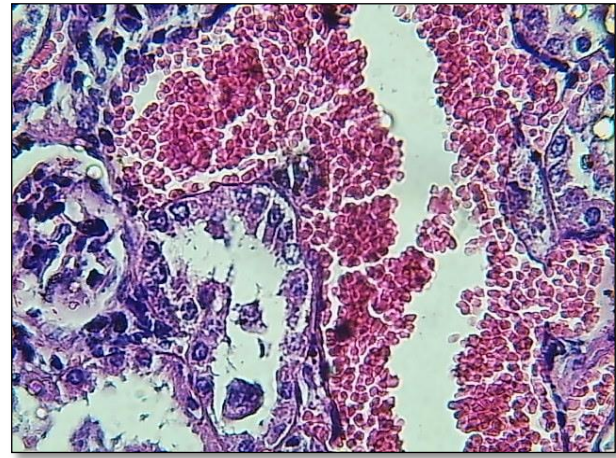


Figure 13: section of renal cortex (treated) shows severe necrosis of lining cells of renal tubules with intra luminal cellular desquamation and marked interstitial hemorrhage. H&E stain, 40x.

- Spleen (control versus treated)

The spleen (control) also demonstrated normal histoarchitecture, with well-defined white and red pulp regions and intact lymphoid follicles (**Figures 14 and 15**). While splenic sections in treated group demonstrated mild white pulp atrophy with follicular hypoplasia, accompanied by severe sinusoidal congestion and red pulp distention (**Figure 16**). The red pulp also showed edema and hypoplasia of splenic cords (**Figure 17**), indicating impaired splenic structural integrity.

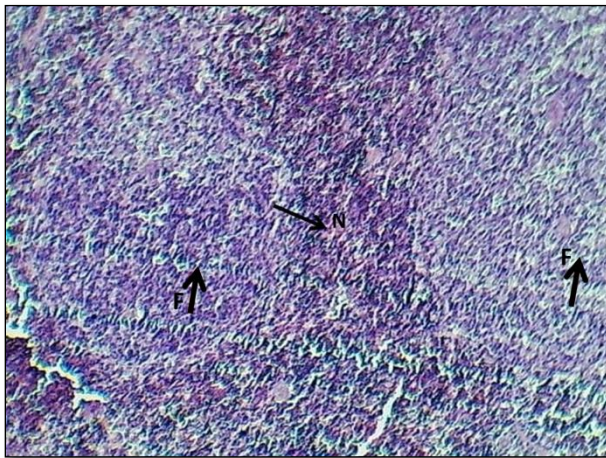


Figure 14. Section of spleen (control) shows normal appearance of splenic white pulp follicles (F) & normal sinusoidal red pulp (N). H&E stain, 10x

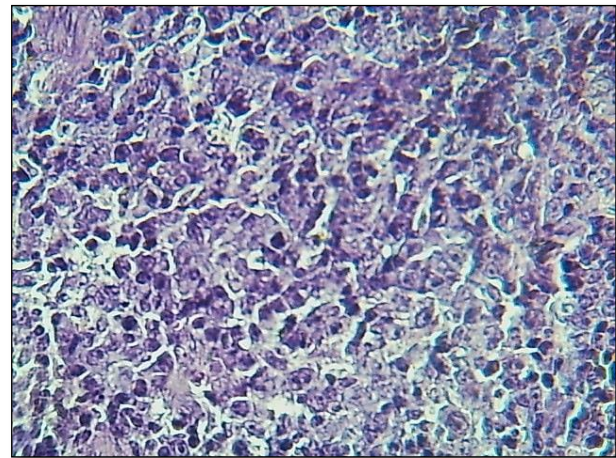


Figure 15. Section of spleen (control) shows normal cytoarchitecture of splenic white pulp lymphoid follicles. H&E stain, 40x

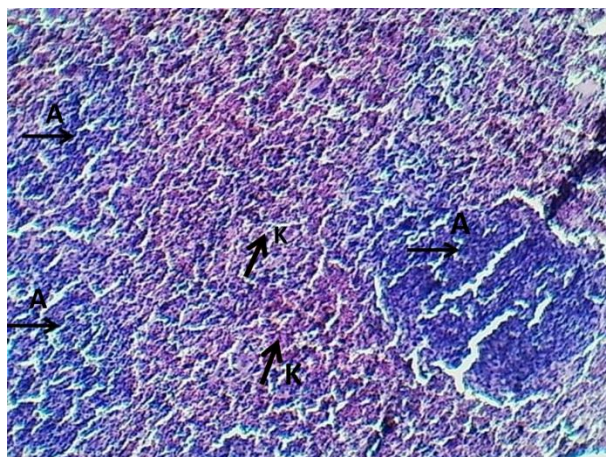


Figure 16: section of spleen (treated) shows mild atrophy associated with hypoplasia of splenic white pulp follicles (A) & severe congestion with distention of sinusoidal red pulp (K). H&E stain, 10x

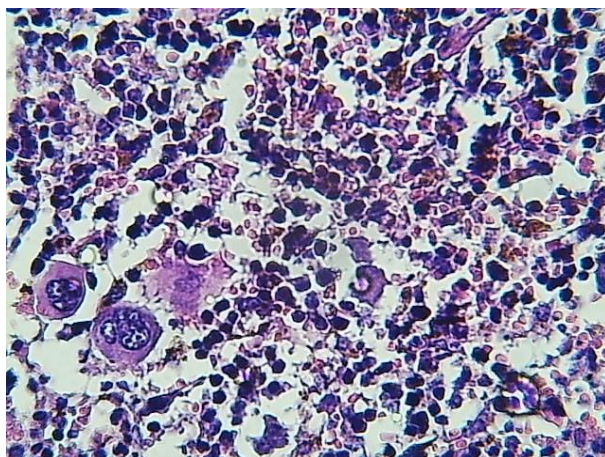


Figure 17. Section of splenic red pulp (treated) shows marked congestion with edema and hypoplasia of splenic cords. H&E stain, 40x.

4. Discussion

The results of qualitative analysis are in confirmation with a previous report, which highlighted an important role of phenolic and flavonoid on the free-radical scavenging ability of broccoli²⁹. Glucosinolates were also more abundant and the dominant family among the bioactive compounds that could be quantified. Biological effects highlighted are detoxification, anti-inflammatory reactions, chemoprevention, as well as cardiovascular and microbial disease shield³⁰. Tannins and alkaloids were found to be present in moderate levels, which may also have some antibacterial, antioxidant, and enzyme-inhibitory effects. Glycosides (other than glucosinolates) were found in lower, yet biologically significant ratios, having cardioprotective and anti-inflammatory action³¹. Saponins had the lowest concentration but still possess the capacity to control cholesterol metabolism and cause tumor cell cytotoxicity³². In general, the phytochemical profile of broccoli extract work together to presented multiple health benefits such as the antioxidant, anti-inflammatory, anticancer and antimicrobial abilities. Variation in these compounds may be affected by broccoli variety as well as growth conditions, harvesting time and plant part³³.

The result of hematological analysis indicates that the broccoli extract may didn't produce overt hematological toxicity under experimental condition. These results may align with previous studies that indicated the cruciferous vegetables may have hemato-protective effects of in animal models³⁴. There were slight but non-significant increases in platelet numbers of the treated group. The level of PT did not change, and PTT was slight but non significantly increased after dosed compared with controls. This result may suggest a possible anticoagulant-like effect of broccoli extract. This may align with study that found the broccoli extract may increase clotting time through the intrinsic system and lower blood viscosity by increasing lipid metabolism and vascular function. The slight prolongation may be association with sulfur-containing substances such as sulforaphane and isothiocyanates, which have been reported to influence coagulation pathway and may mildly inhibit of coagulation factors VIII, IX, XI, and XII³⁵. In contrast, other study found that the broccoli is rich in vitamin K, which promotes prothrombin synthesis and enhances coagulation³⁶. Therefore, the net hematological outcome depends on the type of extract, concentration of active compounds, and dosage, explaining the variable coagulation responses reported in different studies. However additional investigation such as coagulation factor assay, thrombin time, fibrinogen measurement are required to approved this activity.

Biochemical analysis revealed that the broccoli extract did not significantly affect total serum cholesterol levels in the treatment group under specified conditions (dose, duration, health status). This outcome corresponds with research³⁷, which indicates consuming broccoli did not significantly change total cholesterol levels in mice on a high-fat, high-cholesterol diet. Additionally, not all plant extracts affect total cholesterol. A three-week study on rats comparing medicinal plant extracts (Piper cubeba fruit, Physalis angulata flower, Rosa hybrida flower) found no significant differences in total or LDL-cholesterol levels, while HDL values increased in one group³⁸. The unchanged cholesterol levels in this study do not contradict previous findings but are explained by differing experimental conditions. Significant reductions in total and LDL cholesterol have been mainly reported in metabolic disorder models, such as diabetic mice or when using high-glucoraphanin broccoli, where lipid-lowering effects are more pronounced under metabolic stress, with higher doses and longer treatment periods^{39,40}. However, the lack of lipid changes despite mild liver histological alterations indicates that cholesterol may represent relatively late-responding biomarker, as noted by⁴¹, who showed that hepatic structural and oxidative changes may occur before shifts in circulating lipids. The use of healthy mice, a relatively low dose, and a short duration likely maintained lipid homeostasis, which may explain the minimal effect observed.

Broccoli extract significantly reduced TSH and elevated T4 with non-significant elevating in T3. These results may indicate a possible modulatory effect on thyroid activity and secretion of thyroid hormones through a negative feedback mechanism that suppresses the release of pituitary TSH. Study found the bioactive chemicals like sulforaphane have antioxidant activity, which may enhance thyroid hormone production by reducing oxidative stress⁴². Recently, several studies indicate that brassica plants didn't adversely affect for thyroid function in humans and animals with sufficient iodine status⁴³. However, another study⁴⁴, found that allyl isothiocyanate lowered T3 and T4 in diabetic rats, showing that pathological circumstances or metabolites can have contrasting effects. These variations are possibly dependent upon extract composition, dose, and iodine levels. Further investigation involving different doses, longer treatment period, measurement of oxidative stress markers and inflammatory biomarkers, thyroid related enzymatic activity are recommended.

The phytochemical content of broccoli extract may be associated with histopathological changes even in the absence of significant hematological toxicity. Although there is no significant change in hematological and biochemical parameters, these histological findings may indicate early organ stress or mild tissue injury at the administered dose. This result suggests that morphological alterations may occur before systemic symptoms. The FDA recommendations for biomarker qualification specify that "temporal differences in dynamic change can be demonstrated at separate time points if the temporal relationship of a marker to drug effect has been adequately characterized." The relationship between the temporal initiation of biomarker changes of a biomarker and a histopathologic change defines biomarker sensitivity; often morphological changes may occur before individual testable biomarker changes can be detected⁴⁵. However, there is a limitation in the interpretation of histopathological alteration and warrant further investigation due to the absence of biochemical markers such as liver enzymes (ALT, AST) and renal function indicators (creatinine, urea). Furthermore, the observable tissue changes may be partially associate with the stimulation of thyroid hormone induced by broccoli extract. As previously demonstrated in⁴⁶, increased thyroid hormones (T₃& T₄) are known to enhance oxidative metabolism and stimulating ROS production which may contribute to oxidative stress and cellular damage in several organs. In contrast, several studies demonstrated that broccoli extract exert significant protective effects against toxic insults such as arsenic, providing significant tissue protection and reducing the histopathological changes induced by this toxin⁴⁷.

Although broccoli extract and its bioactive compounds are broadly known for their antioxidant, hemato-protective, and potential anticoagulant properties, the present findings suggest that

these advantageous effects may also be accompanied by some biological differences at the tested dose. Histological examination revealed adverse tissue changes that may indicate early oxidative stress or mild tissue toxicity.

In addition, the effect on metabolic and endocrine activities may contribute to tissue-related side effects under normal physiological conditions. Therefore, careful evaluation of dosage, duration of exposure, and tissue integrity remains essential before considering long-term biological applications of broccoli extract

5. Conclusion

This study shows that the Broccoli extract has non-significant hematological alteration; it did not influence WBC, Hb, PCV, or platelet counts. A slight prolongation in aPTT may show a possible anticoagulant effect, presumably due to sulfur-containing phytochemicals that effect on intrinsic coagulation. The substance may be modulating the thyroid activity by reducing TSH, non-significant increase in T3 and increase T4 levels. Total cholesterol is less susceptible to diet than hematological or hormonal indicators. The normal physiological status animal and short treatment duration may explain identical total cholesterol amounts. Histological examination indicates presence of hepatic and renal tissue congestion, minor necrosis, and inflammatory cell infiltration. These alterations may indicate early oxidative stress or mild tissue toxicity under tested dose. However, doses beyond 200 mg/kg body weight of the extract may generate negative effects and require dose selectivity and long-term safety testing. Further long-term experiments with wider dose range are needed to evaluate broccoli extract's dose–response relationship, chronic effects to find novel therapeutic uses.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Clearance

All experimental techniques were performed in alignment with the OECD guidelines NO.407 in the Biotechnology Research Center, Al-Nahrain University, and approved by the institutional ethical committee (CSEC/1025/010; approved on 7 October 2025).

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