African Journal of Biological Sciences

Research Paper Open Access

Purification and Characterization of Cyclooxygenase (Cox1) Enzyme Extracted from Patients with Hyperthyroidism

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Article Info

Volume 6, Issue 8, April 2024
Received: 14 Feb 2024
Accepted: 05 March 2024
Published: 07 April 2024

Abstract

Background Hyper the most common disorders of the endocrine system worldwide and the cyclooxygenase cox1 which is responsible for biosynthesis of prostanoids, including thromboxane and prostaglandins, from arachidonic acid. **Objectives** This study aimed at extraction and purification of cyclooxygenase from patients with hyperthyroidism. **Material and method** Serum is separated and subjected to a series of purification processes including: precipitation by ammonium sulfate, filtration by centrifugation radiator, dialysis in presence of ammonium bicarbonate, separation using the technology of ion exchange, lipholization and then estimating approximate molecular weight of the enzyme using gel filtration technique. **Result** Our investigation revealed heightened COX-1 enzyme activity in hyperthyroidism (1.95±0.09ca) compared with the control (1.08±0.02b) and the best method for extracting the enzyme was gelfiltration where the specific activity (U/ mg) is (23) and the purification folds is (10.2). Notably, our study also unveiled the potential of the Conocarps plant extract in inhibiting COX-1 enzyme activity by Alcohol and Aqueous extract with concentration 5% where Remaining activity is (85.8), suggesting promising avenues for further research in thyroid-related therapeutic interventions. **Conclusion** The enzyme was extracted from patients with hyperthyroidism.

Key words: Cyclooxygenase-1, Hyperthyroidism, Purification

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Introduction

The Cyclooxygenase (COX, also called prostaglandin H synthase or PGHS). This enzyme has two activities, a cyclooxygenase and peroxidase thereby responsible for catalyzing the conversion of arachidonic acid into prostaglandin G2, which are further peroxidised to prostaglandins H2, the precursor of molecules, such as prostaglandins, prostacyclin, and thromboxanes.[1] COX exists in isoforms, COX-1is known to be a constitutive enzyme that is responsible for the normal physiological functions including maintenance of the integrity of the gastric.

first purified in 1976 and cloned in 1988, is the key enzyme in the synthesis of prostaglandins (PGs) from arachidonic acid. In 1991, several laboratories identified a product from a second gene with COX activity and called it COX-2. However, COX-2 was inducible, and the inducing stimuli

included pro-inflammatory cytokines and growth factors, implying a role for COX-2 in both inflammation and control of cell growth.[2]

Hyperthyroidism (overactive thyroid) happens when your thyroid produces and releases more thyroid hormones than your body needs. This causes aspects of your metabolism to speed up. Approximately 1 out of 100 people over the age of 12 have hyperthyroidism in the United States. It is treatable high enzyme activity leads to high levels of hyperthyroidism

Causes of hyperthyroidism include [3]

Graves' disease, an autoimmune condition.

- Thyroid nodules.
- Thyroiditis (inflammation of the thyroid).
- Postpartum thyroiditis (inflammation of the thyroid that happens after giving birth).
- Excess iodine in your blood from diet and/or medication.
- Over-treatment of hypothyroidism through medication.
- A benign (noncancerous) tumor in your pituitary gland.

Materials and Methods

Collection of Blood and serum preparation

Three ml of blood were obtained from each subject by vein puncture. put into disposable tube containing separating gel.the blood in the gel containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at3000×g for approximately 5 minutes then the sera were obtained and stored at -20˚C until analysis.the samples may be stored frozen at -20˚C for 3 months showed no performance difference.

The patient and control group

The group who subjected to this study were (75) persons in the age group ranging from 14-82 years,this group comprised of Hyperthyroidism 45 divided into male 9 and female 36 and control 30 All of those patient were admitted to comprehensive from including the following :age,gender,past history of disease and medication.

T3, T4, TSH hormone test

Using the Japanese (Tosoh AIA) device to measure the T3, T4 and TSH hormone.use T3, T4, TSH Kit.

protein assay solution

By using Brad fort method [4]

Enzyme assay used ELISA KIT

Use Human cyclooxygenase -1, cox-1 ELISA KIT

purification of cyclooxygenase enzyme

There were 4 steps for purification of enzyme

Ammonium sulfate precipitation.

Place the sample inside the beaker containing a magnetic stirrer, then it is placed inside a larger beaker containing water and ice, then slowly add saturated ammonium sulfate (7.3g) to hyperthyroidism samples and 5.9g to hypothyroidism samples bring the final concentration to 50% saturatio

Volume of ammonium sulfate needed is equal to the volume of sample

Adding the ammonium sulfate very slowly it takes three hours ensures that local concentration around the site of addition does not exceed the desired salt concentration. Then centrifuged at 9,000 rpm for 15 minutes at 4˚C.

Dialysis−2

The sediment is taken after sedimentation with ammonium sulfate and placed in a dialysis bag volume is 3500, then place in water bath 37 ˚C for 10 minutes.

Purification by ion exchange chromatography

Preparation of Buffers and Solutions

Sodium chloride solution (0.25M)

Sodium chloride (1.46 g) was dissolved in suitable volume of D.W and the volume was complete to 100 ml with D.W.

Sodium hydroxide solution (0.25M)

Sodium hydroxide (0.9 g) was dissolved in suitable volume of D.W and the volume was complete to 100 ml with D.W.

Hydrochloric acid (HCl; 0.25 M)

It was prepared by adding 0.9 ml of concentrated HCl to a glass bottle and then volume was completed to 100 ml of D.W.

Tris-HCl buffer (0.05 M, pH 7)

This buffer was prepared by dissolving 0.6 g of Tris-HCl in 90 ml of D.W, pH was adjusted to 7 and the volume was completed to 100 ml with D.W.

Potassium phosphate buffer (0.5M; pH: 7)

This buffer was composed of two types of solutions:

- Solution A:

Dipotassium hydrogen phosphate (K2 HPO4) was prepared by dissolving 8.7 g in 100 ml distilled water (D.W).

- Solution B:

Potassium di hydrogen phosphate (KH2 PO4) solution was prepared by dissolving 6.8 g in 100 ml D.W. Then, 61 ml of K2 HPO4 was mixed with 39 ml of KH2 PO4 solution, pH was adjusted to 7.0 and the volume was completed to 200 ml with D.W.

Ion exchange chromatography

Diethyl-amino-ethyl cellulose (DEAE-cellulose) was prepared according to Whitaker (1972) as follow: DEAE-cellulose resin (20 g) was suspended in 1 liter of D.W. then beads left to settle down and washed several time with D.W until getting clear appearance. The suspension was filtered by Whatman No. 1 filter paper using buchner funnel under discharging. Then the resin was re-suspended in sodium chloride solution (0.25M) and sodium hydroxide solution (0.25M), respectively. The suspension was filtered again and washed several times by hydrochloric acid (HCL; 0.25M) and next by D.W before it was equilibrated with Tris-HCl buffer (0.05M; pH 7). Then the resin was packed into column (3 × 20 cm). Enzyme solution was then added to DEAE-cellulose column. After that, the column was washed with an equivalent volume of the same buffer and attached proteins were stepwise eluted with gradual concentrations of sodium chloride (0.1- 1M). The flow rate through the column was 3 ml and the absorbance of each fraction was measured at 280 nm using UV-visible spectrophotometer

Purification by gel filtration chromatography

Sephadex G-150 was prepared as recommended by Pharmacia Fine Chemicals Company. A suitable amount of Sephadex G-150 was suspended in phosphate buffer (pH 7), subjected to heating at 90°C for 5 hrs to ensure beads swelling then, degassed and packed in a glass column (2 × 40 cm) and equilibrated with phosphate buffer. A purified enzyme obtained from ion exchange step was applied onto the matrix. Then elution was achieved at a flow rate of 3ml/fraction using the same buffer for equilibration. After that, the absorbance of each fraction was also measured at 280.

Phosphate buffer (0.05M,Ph7)

This buffer was prepared by dissolving 1.15g Na2HPo4 in 1000ml of D.W.

Results and Discussion

That as shown in table 1 there are significant differences in the levels of thyroid hormones between patients and healthy people, as we note that the T3 hormone has decreased significantly(0.72±0.05)in patient with hypothyroidism and also increased significantly (1.95±0.09)in patients with hyperthyroidism compared to healthy subjects Figure (1), as we note that the T4 hormone has decreased significantly(4.06±0.19)in patient with hypothyroidism and also increased significantly (13.09±0.24)in patients with hyperthyroidism compared to healthy subjects Figure (2)and we note that the TSH hormone has increased significantly(48.54±5.68)in patient with hypothyroidism and also decreased significantly (0.03±0.004)in patients with hyperthyroidism compared to healthy subjects Figure(3).

Table (1): Mean differences in Thyroid hormones between control and patients.

Parameters	Control	Hypo	Hyper
Groups	Mean±S.E		
T3	1.07±0.02 b	0.72±0.05 a	1.95±0.09 c
T4	7.70±0.11 b	4.06±0.19 a	13.09±0.24 c
TSH	2.46±0.17 b	48.54±5.68 c	0.03±0.004 a

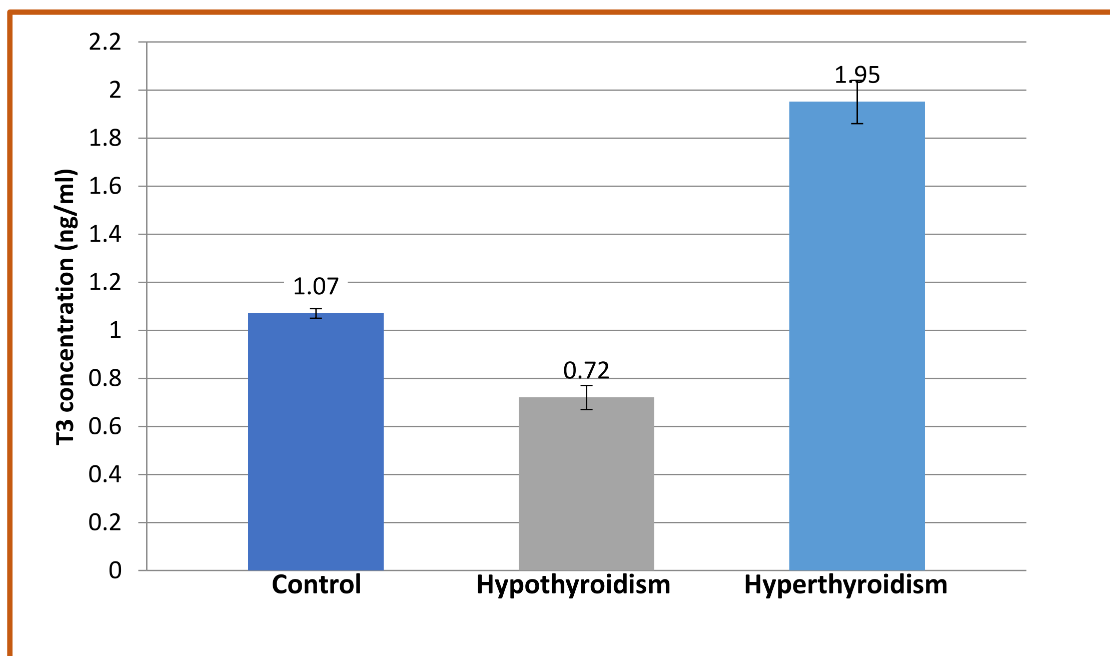
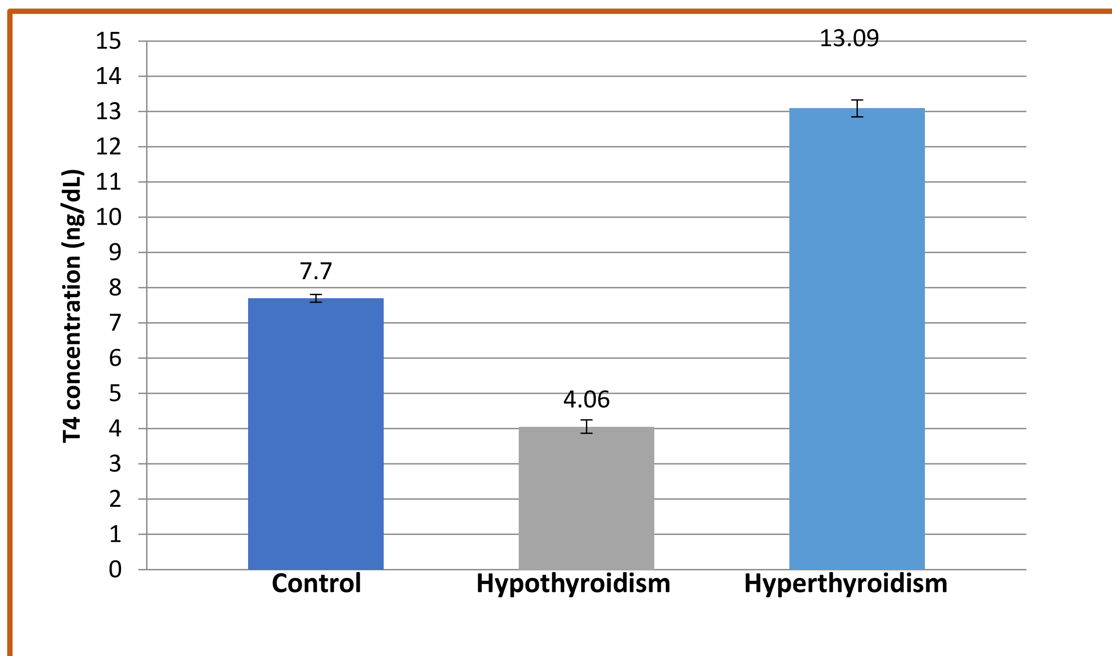


Figure (1): Mean differences in T3 hormone between control and patients.



That as

Figure (2): Mean differences in T4 hormone between control and patients.

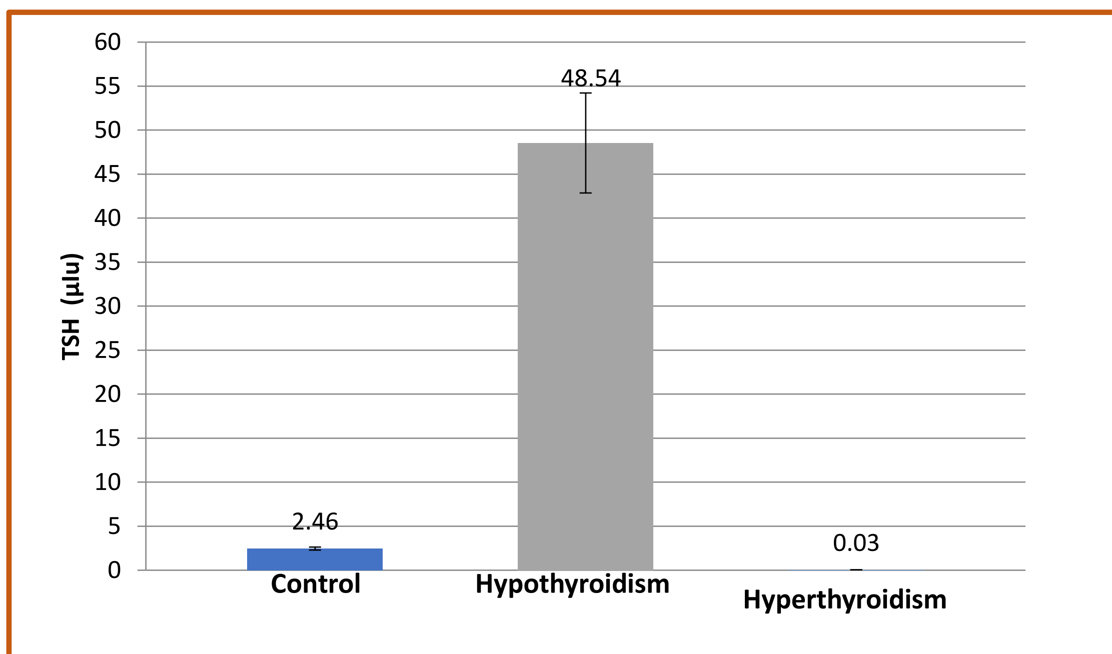


Figure (3): Mean differences in TSH hormone between control and patients.

In the table 2 That as shown differences in Thyroid hormones between control and patients according to gender.that with regard to the T3 hormone, there are significant differences for males, as well as for females. As for the comparison in disease, we notice that the level of T3 hormone differed significantly between males and females in significantly in hyperthyroidism patients p-value (0.452).in the T4 hormone, there are significant differences for males, as well as for females. As for the comparison in disease, we notice that the level of T4 hormone differed significantly between males and females in hypothyroidism patients p- value (0.042), and it did not differ significantly in hyperthyroidism patients p-value (0.733),and in the TSH hormone, there are significant differences for males, as well as for females. As for the comparison in disease, we notice that the level of TSH hormone differed significantly between males and females in

hypothyroidism patients p- value (≤0.0001), and it did differ significantly in hyperthyroidism patients p-value (0.046).

Table (2): Mean differences in Thyroid hormones between control and patients according to gender.

Parameters		Control	Hypo	Hyper
Groups		Mean±S.E		
T3	Male	1.06±0.11 b	0.51±0.03 a	1.99±0.21 c
	Female	1.20±0.04 b	0.76±0.05 a	1.94±0.33 c
p-value			0.012*	0.452
T4	Male	7.53±1.11 b	3.40±0.63 a	12.61±1.42 c
	Female	7.87±0.54 b	4.18±0.22 a	13.19±1.09 c
p-value			0.042*	0.733
TSH	Male	2.36±0.25 b	74.66±4.22 c	0.04±0.01 a
	Female	2.56±0.16 b	43.79±5.30 c	0.03±0.01 a
p-value			≤0.0001**	0.046*

In the table (3) Mean differences in thyroid hormones between control and patients according to age That as shown in table that there is a significant difference in the T3 hormone in different age groups, and the highest value of T3 was in the age group(14-30) of hyperthyroidism patients(2.09±0.7), and the lowest value of T3hormone was in the age group(67-82) of hypothyroidism patients(o.6±0.05). there is a significant difference in the T4 hormone in different age groups, and the highest value of T4was in the age group(67-82) of hyperthyroidism patients(15.15±1.9), and the lowest value of T4hormone was in the age group(49-66) of hypothyroidism patients(3.00±0.6).),and in TSH hormone there is a significant difference in the TSH hormone in different age groups, and the highest value of TSH hormone was in the age group(67-82) of hypothyroidism patients(73.12±6.6), and the lowest value of TSH hormone was in the age groups(31-48)and(49-66) of hyperthyroidism patients(0.02±0.001).

Table (3): Mean differences in Thyroid hormones between control and patients according to age.

Parameters	Age (year)	Control	Hypo	Hyper	LSD(0.05) (A*B)
Groups		Mean±S.E			
T3	14-30	1.05±0.3	0.86±0.15	2.09±0.7	0.171
	31-48	1.14±0.2	0.71±0.11	1.86±0.2	
	49-66	0.90±0.1	0.58±0.04	2.04±0.3	
	67-82	1.16±0.3	0.62±0.05	2.04±0.2	
T4	14-30	7.62±1.2	4.19±0.5	10.77±1.1	0.985
	31-48	7.66±1.4	4.24±0.2	12.91±1.3	
	49-66	9.70±0.8	3.00±0.6	14.09±2.1	

	67-82	8.29±1.3	3.80±0.4	15.15±1.9	
TSH	14-30	2.50±0.4	49.52±8.2	0.06±0.001	7.656
	31-48	2.33±0.1	42.73±6.4	0.02±0.001	
	49-66	2.93±0.6	69.75±7.3	0.03±0.002	
	67-82	2.41±0.3	73.12±6.6	0.02±0.001	

In the table (4) Pearson correlation coefficient among parameters in hypothyroidism patients. That as shown in table that there are correlations between thyroid hormones in hypothyroidism in T3and T4

A positive direct significant relationship(r=0.468) at(p≤0.05) figure(4), as well as a significant negative inverse relationship between T3andTSH hormone(r=-0.635)figure(5), and there was a significant inverse relationship between T4and TSH hormone(r=-0.453), while the rest of the relationships were not significant or influential

Table (4); Pearson correlation coefficient among parameters in

Correlations				
		T3	T4	TSH
age	r	-.280-	-.119-	.157
	Sig.	.084	.472	.340
	r	1	.468**	-.635-**
	Sig.		.003	.000
T4	r		1	-.453**
	Sig.			.004

**. Correlation is significant at the 0.01 level (2-tailed).

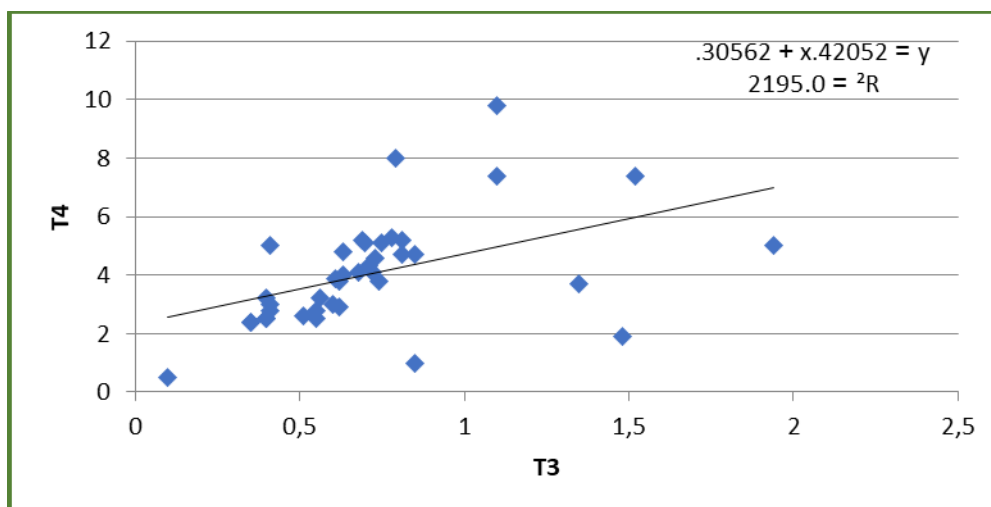


Figure (4): Significant difference between T3 and T4 in hypothyroidism patients.

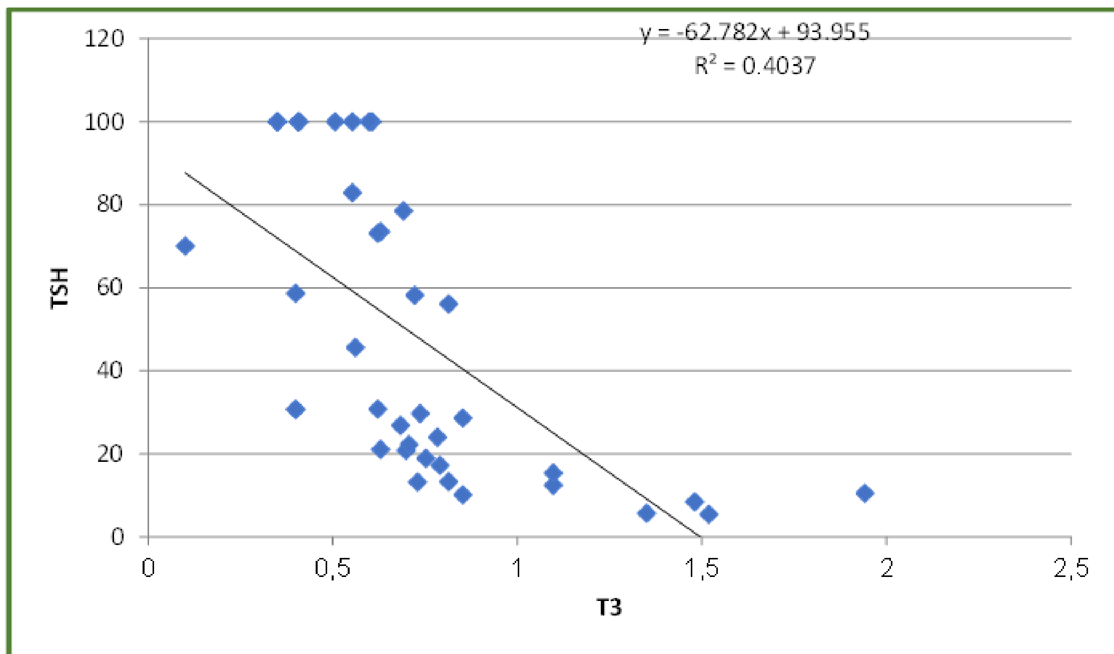


Figure (5): Significant difference between T3 and TSH in hypothyroidism patients.

In the table (5) person correlation coefficient among parameters in hyperthyroidism patients.That as shown in table that there are correlations between thyroid hormones in hyperthyroidism in T3and T4hormone, as A positive direct significant relationship(r=0.543) at($p \leq 0.05$), as well as un significant negative inverse relationship between T3andTSH hormone(r=-0.021), and there was un significant inverse relationship between T4and TSH hormone(r=-0.132), while the rest of the relationships were not significant or influential figure(6,7)

Table (5): Pearson correlation coefficient among parameters in hyperthyroidism patients.

Correlations				
		T3	T4	TSH
Age	r	.032	.208	-.220-
	Sig.	.835	.175	.151
T3	r	1	.543**	-.021-
	Sig.		.000	.894
T4	r		1	-.132-
	Sig.			.392

**. Correlation is significant at the 0.01 level (2-tailed).

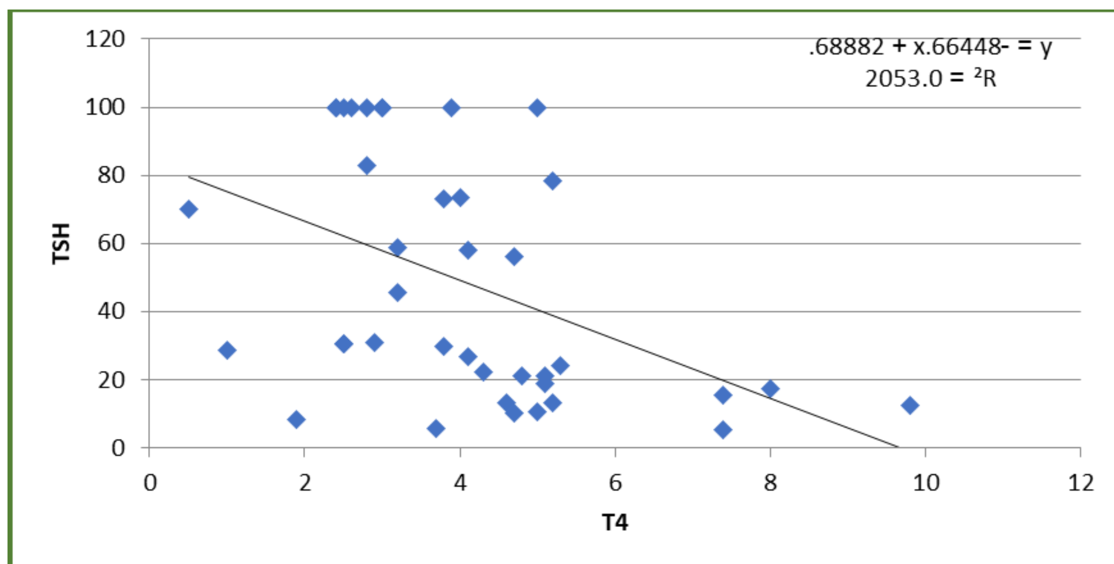


Figure (6): Significant difference between T4 and TSH in hypothyroidism patients.

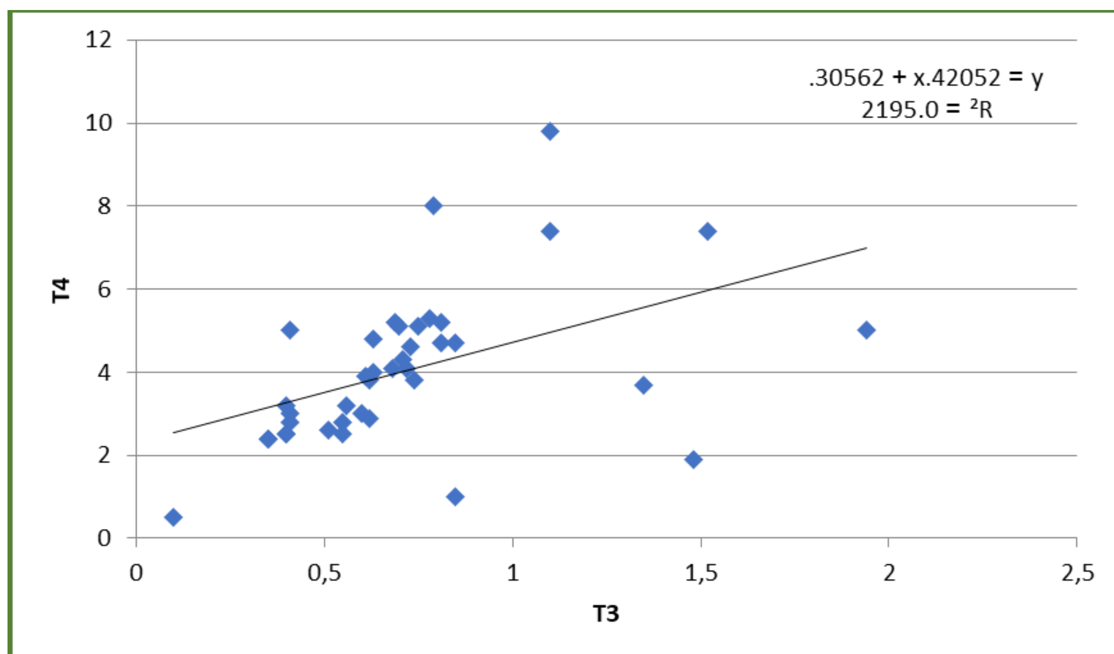


Figure (7): Significant difference between T3 and T4 in hyperthyroidism patients.

Purification of enzyme

In this study, we successfully purified the COX-1 enzyme using a series of chromatographic techniques. As shown in Table 6, the purification process involved several steps, including ammonium sulfate precipitation, DEAE-cellulose ion exchange chromatography, and Sephadex-G150 gel filtration chromatography. At each step, we calculated the specific activity, total activity, purification fold, and yield of the enzyme using the provided equations.

Our results demonstrate that the specific activity of the COX-1 enzyme increased with each purification step, reaching a maximum value of 23.3 U/mg after gel filtration. This suggests that the enzyme became more pure and catalytically efficient as impurities were removed during the purification process. However, we also observed a decrease in total activity with each purification step, resulting in a final yield of 40.8% after gel filtration. This indicates that some of the enzyme was lost during the purification process.

After dialysis, we concentrated the enzyme using sucrose and applied it to a DEAE-cellulose ion exchange column. We then concentrated the peak of enzyme activity eluted from the ion exchange column andapplied it to a Sephadex-G150 gel filtration column for further purification

Table (6): purification step

purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	45	0.8	0.35	2.28	36	1	100
Ammonium sulphate precipitation 50%	10	1.8	0.3	6	18	2.63	50
DEAE-cellulose	18	0.9	0.1	9	16.2	3.94	45
Sephadex-G150	21	0.7	0.03	23.3	14.7	10.2	40.8

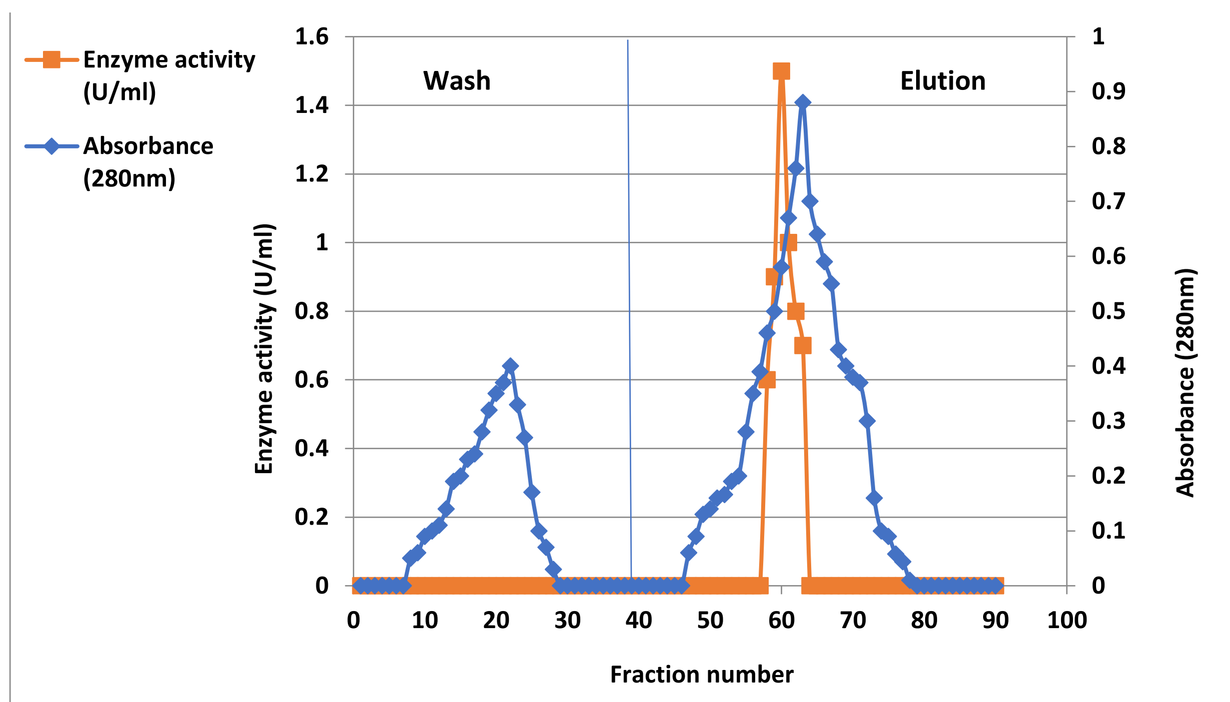


Figure (8) Ion exchange chromatography on DEAE cellulose column

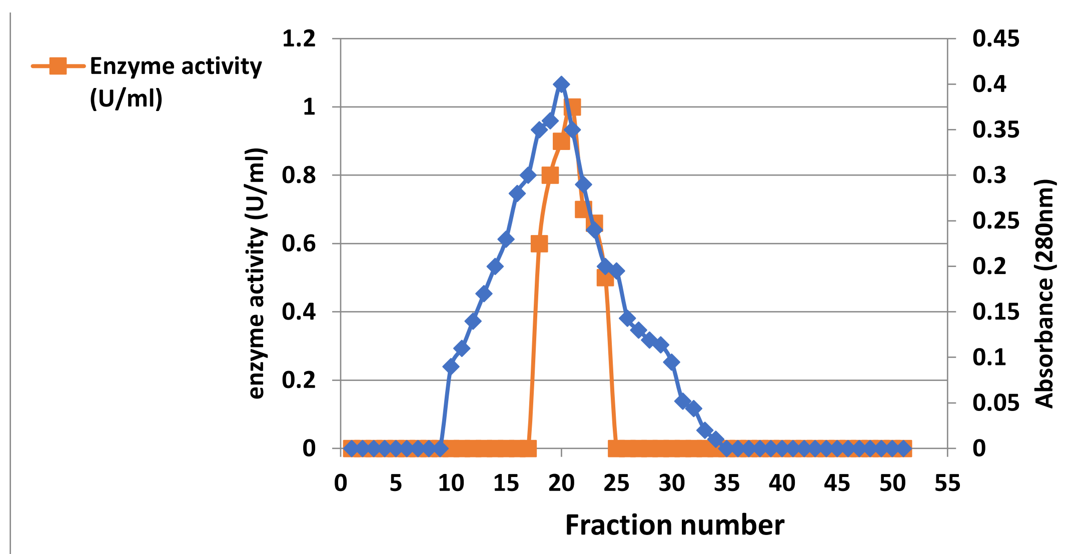


Figure 9 cox1 activity and absorbance at 280 nm as afunction of fraction Number during gel filtration chromatography on Sephadex G150

Enzyme characterization of COX-1: pH, temperature and molecular weight effects

Impact of temperature on COX-1 enzyme activity

Our study conducted experiments to assess the impact of temperature and COX-1 enzyme, testing a range from 32°C to 52°C. The data in Table 8 reveals a pronounced correlation between temperature and enzyme activity. At 32°C and 37°C, the enzyme displayed robust activity, with 100% of the initial activity retained. This suggests a high level of stability and functionality within this temperature range.

As the temperature increased to 42°C, a slight reduction in enzyme activity was observed, with 90% of the initial activity remaining. This indicates that the enzyme remained relatively stable but began to show signs of decreased activity.

Subsequently, at 47°C, there was a more significant drop-in enzyme activity, with only 80% of the initial activity remaining. This temperature represents a critical point at which the enzyme's stability starts to deteriorate noticeably.

The most substantial decline in enzyme activity was observed at 52°C, with only 70% of the initial activity remaining. This temperature marked a significant departure from the enzyme's optimal conditions, resulting in a significant decrease in its catalytic effectiveness.

In summary, temperature exerts a substantial influence on enzyme activity. The data highlights the critical importance of maintaining an appropriate temperature range when working with enzymes, as deviations from the optimal temperature can lead to a diminishing enzyme activity, potentially affecting the outcome of biochemical processes (figure10 is a visual representation of the data in the table 7)

Table 7 Temperature-Dependent COX-1 Enzyme Activity

Remaining activity (%)	Temparature (°C)
100	32
100	37
90	42
80	47
70	52

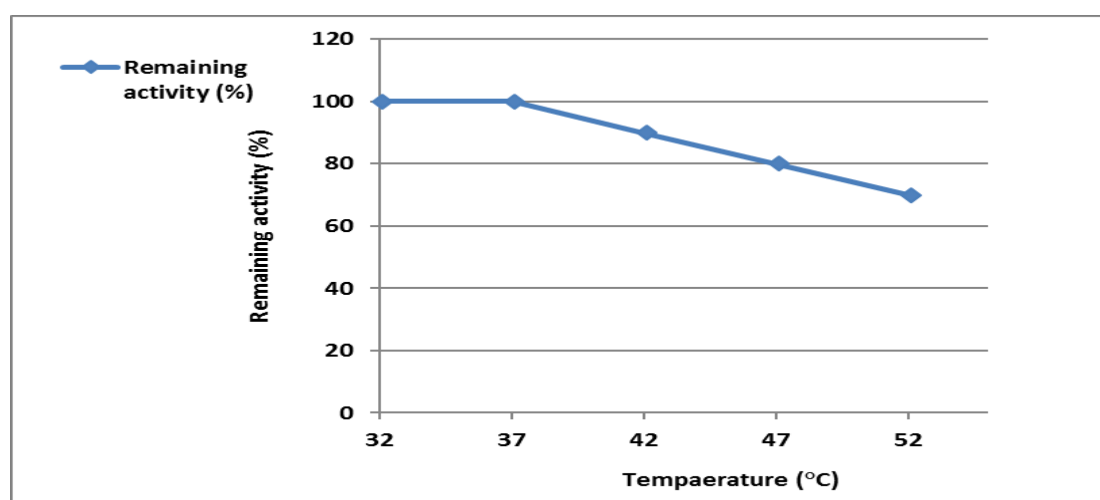


Figure 1 Temperature-Dependent Remaining Activity of COX-1 enzyme

Impact of initial medium pH on COX-1 enzyme activity

To assess the effect of pH on COX-1 enzyme activity, experiments were conducted in a controlled environment. The pH of the medium was adjusted to pH 5, pH 6, pH 7, pH 8, and pH 9, and the remaining enzyme activity was measured as a percentage of the initial activity at each pH level.

The data in Table 9 elucidates a strong connection between the initial medium pH and COX-1 enzyme activity:

At pH 5, the enzyme exhibited reduced activity, with only 50% of the initial activity remaining. This lower pH level appears to hinder enzyme function. As the pH increased to 6, the enzyme's activity improved significantly, with 80% of the initial activity retained. The most remarkable finding was at pH 7, where the enzyme displayed its optimum activity, with 100% of the initial activity maintained. At this pH, the enzyme's specific activity reached 0.644 U/mg protein, indicating peak functionality. Beyond the optimum at pH 8, the enzyme activity remained relatively high, with 90% of the initial activity retained.

However, at pH 9, the enzyme activity experienced a noticeable decline, with only 70% of the initial activity remaining, indicating reduced functionality at this alkaline pH.

This experiment demonstrates the substantial influence of initial medium pH on COX-1 enzyme activity. The optimum pH for COX-1 activity was identified at pH 7, where the enzyme exhibited its highest specific activity. Remarkably, the enzyme's activity gradually increased as the pH was elevated from acidic to the optimum pH but declined when moving to more alkaline conditions (figure 11 is a visual representation of the data in the table8).

Table 8 Effect of pH on COX-1 Enzyme Activity

Remaining activity (%)	pH
50	5
80	6
100	7
90	8
70	9

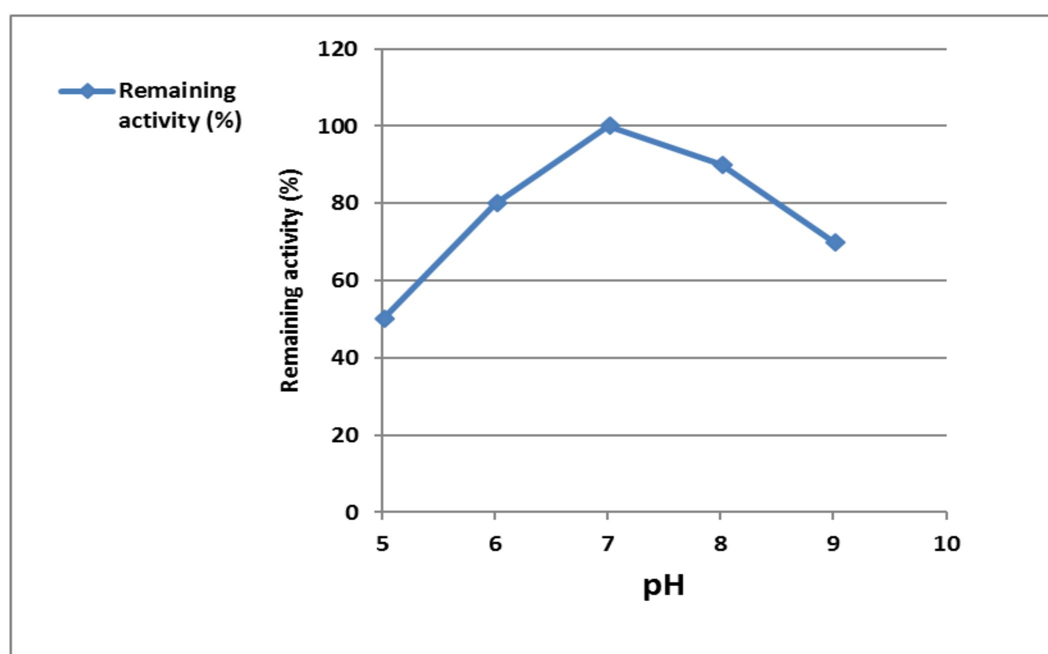


Figure 2 pH-Dependent Variation in Remaining Activity of COX-1 Enzyme

Determination of COX-1 Enzyme Molecular Weight using Gel Filtration Chromatography

In this study, we utilized gel filtration chromatography to determine the molecular weight of the COX-1 enzyme. The chromatography was performed using a Sephadex G-150 column, which was equilibrated with a 0.05M phosphate buffer at pH 7. The elution was carried out with the same buffer.

The molecular weight of the COX-1 enzyme was determined by comparing its Ve/Vo ratio with those of known standard proteins. These standard proteins, which served as molecular weight markers, included alcohol-dehydrogenase (with a molecular weight of 150,000), albumin (with a molecular weight of 66,000), carbonic anhydrase (with a molecular weight of 29,000), and lysozyme (with a molecular weight of 14,300). The Ve/Vo ratio for each standard protein was calculated from their elution volumes, which were measured at 280 nm with a UV-Vis Bio-Rad spectrophotometer.

the results were plotted on a graph showing the relationship between molecular weight and the ratio of Ve/Vo for each protein. The graph provided a visual representation of the data, allowing us to determine the molecular weight of the COX-1 enzyme based on its position on the graph relative to the standard proteins. By knowing that the COX-1 enzyme has a Ve/Vo ratio of 2.4, we were able to determine that its molecular weight is 75,000 (Table 9 and figure12).

In conclusion, gel filtration chromatography proved to be an effective technique for determining the molecular weight of the COX-1 enzyme in this study

Table 9 Molecular Weight and Ve/Vo Ratios of Standard Proteins and COX-1 Enzyme

Ve/Vo	Molecular weight
1.4	150
1.4	150
2.4	75
2.6	60
3.1	29
3.3	14

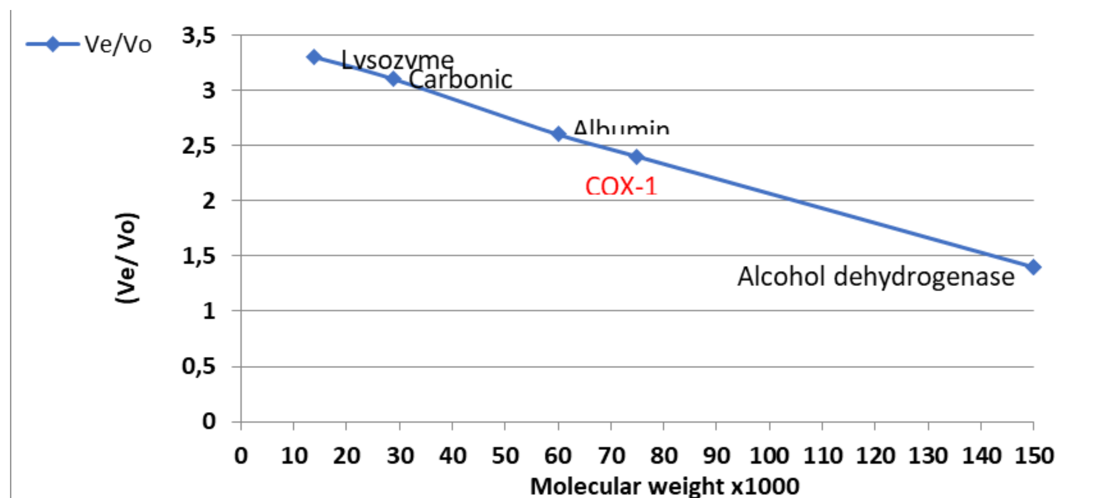


Figure3 Determination of COX-1 Enzyme Molecular Weight using Gel Filtration Chromatography

Our study results illuminate the significant disparities in thyroid hormone levels observed among various groups, underscoring the substantial impact of thyroid disorders on an individual's health. Specifically, we examined hypothyroidism, characterized by an underactive thyroid gland resulting in reduced T4 and T3 hormone production, and hyperthyroidism, marked by an overactive thyroid gland leading to excessive T4 and T3 hormone production.

Our findings align with anticipated physiological patterns seen in these conditions. In hypothyroidism, T3 and T4 levels typically decrease, while TSH levels rise as part of the body's compensatory response. Conversely, hyperthyroidism is associated with elevated T3 and T4 levels and reduced TSH levels due to the negative feedback mechanism that suppresses TSH secretion in response to heightened T3 and T4 levels [5].

However, it's crucial to place our findings within a broader context. The study by [6] offers a distinct perspective. This study concluded that thyroid disease is unrelated to gender, obesity, or other comorbidities, underlining the necessity for a comprehensive exploration of diverse factors

influencing thyroid function. This discrepancy illustrates the intricate interplay of variables affecting thyroid health and highlights the multifaceted nature of the gender-thyroid connection

In addition, another article supports our findings, emphasizing the primary influence of age on TSH levels within the context of thyroid function. It concurs that younger individuals within the Hypothyroid group tend to exhibit higher TSH levels, while age does not significantly affect T3 and T4 levels in any of the Control, Hypothyroid, or Hyperthyroid groups [7]

Within this study, a linear regression analysis was conducted to offer a more comprehensive understanding of the relationship between T3 and T4 levels in hypothyroidism patients. The graph shows a positive linear relationship between T3 and T4 levels, with a linear regression equation of $y = 2.4205x + 2.3056$ and an R-squared value of 0.2195. These results align with the Pearson correlation results, which show a positive correlation between T3 and T4 levels in hypothyroidism patients (0.468). Together, these results suggest that there is a significant relationship between T3 and T4 levels in hypothyroidism patients, as demonstrated by both the table4 and the figure 5.

In this study, a linear regression analysis was conducted to offer a more comprehensive understanding of the relationship between T3 and T4 levels in hyperthyroidism patients. The graph shows a negative linear relationship between T3 and T4 levels, with a linear regression equation of $y = 2.4205x + 2.3056$ and an R-squared value of 0.2195 (Figure 7). These results align with the Pearson correlation results, which show a positive correlation between T3 and T4 levels in hyperthyroidism patients (0.543) (Table 5). Together, these results suggest that there is a significant relationship between T3 and T4 levels in hyperthyroidism patients, as demonstrated by both the table and the figure.

The aim of this experiment was to purify and characterize the COX-1 enzyme, which is responsible for biosynthesis of prostanoids, including thromboxane and prostaglandins, from arachidonic acid [8]. Prostanoids are involved in various physiological and pathological processes, such as inflammation, pain, fever, and tumorigenesis [9]. In particular, the COX-1 enzyme has been implicated in thyroid diseases, such as Graves' disease and thyroid cancer [10]. Therefore, understanding the structure and function of the COX-1 enzyme is important for developing therapeutic strategies for various thyroid disorders.

Our study followed a similar protocol to the one reported by, who first isolated and purified the COX-1 enzyme from sheep seminal vesicles. Their protocol involved the use of a series of chromatographic techniques, including DEAE-cellulose, Sephadex G-100, and hydroxyapatite chromatography, to obtain a homogeneous preparation of COX-1. However, we used a different source of the enzyme, which was a crude extract from human cells. We also used different chromatographic techniques and conditions to optimize the purification process.

The purification of the COX-1 enzyme is an important step for studying its role in thyroid diseases. By obtaining a pure and active enzyme, we can perform further experiments to elucidate its mechanism of action, substrate specificity, inhibitor sensitivity, and regulation by various factors in thyroid cells. The purification of the COX-1 enzyme also enables us to compare its expression and activity with those of other isoforms or homologs of the enzyme from different sources or species. Furthermore, the purification of the COX-1 enzyme not only enhances its suitability as a drug discovery target but also aligns with one of our primary objectives: investigating the impact of a plant extract on COX-1 enzyme activity and its relation to hyperthyroidism. Through screening for potential inhibitors or modulators of COX-1 enzyme activity, we aim to identify novel compounds that can effectively modulate prostanoid biosynthesis and influence various thyroid functions

The findings from this experiment shed light on how temperature impacts the activity and stability of the COX-1 enzyme, a crucial player in thyroid diseases. This outcome aligns with a fundamental concept in enzymology: enzymes tend to perform optimally within a specific temperature range, and straying from that range can diminish their effectiveness or even cause them to lose their structural integrity [11]

The objective of this experiment was to explore how pH influences the behavior and stability of the COX-1 enzyme, which plays a crucial role in managing inflammation and pain. Our findings highlight the significant impact of pH on enzyme function, affecting both their structure and their interactions with target molecules. This aligns with a fundamental principle in enzymology, where enzymes perform optimally within a specific pH range, and deviations from this range can impair their function or render them inactive [12].

Conclusion

The purpose of this experiment was to explore the influence of molecular weight on the activity and structure of the COX-1 enzyme, a critical player in managing inflammation and pain. What we uncovered underscores the pivotal role of molecular weight in determining how enzymes operate; it can literally reshape their form and the very place where they interact with their target molecules. This realization dovetails neatly with a fundamental principle in enzymology: enzymes have a preferred molecular weight range where they function at their absolute best, and straying from this range can seriously hinder their performance or even render them ineffective.

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