



Comparative study of the effect of Omega-3 and the nano-loaded Propylthiouracil on thyroxine-induced hyperthyroidism in male white rats

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<https://doi.org/10.59658/jkas.v10i2.1194>

Received: May 04, 2023	Abstract This study was conducted to find out the therapeutic effect of Omega-3 and the zinc oxide nano-loaded propylthiouracil drug in the treatment of thyroxine-induced hyperthyroidism. The results show a significant increased ($p < 0.05$) in serum of Triiodothyronine (T_3) and Thyroxine (T_4) hormones with a decreased Thyroid stimulating hormone (TSH) level in T1 group compared with the control group. On the other hand, the results showed a significant decrease ($P < 0.05$) in (T_3) and (T_4) with increased (TSH) levels in (T2, T3) groups compared to (T1) group. There was a significant ($p < 0.05$) increase in the level of Malondialdehyde (MDA) with a decrease in the level of antioxidants Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) in the (T1) group in comparison with the control group. Also, the results showed a significant ($p < 0.05$) decrease in the level of MDA with an increase in the level of antioxidants (GSH, SOD, CAT) in the (T2, T3) groups as compared to the (T1) group. The results of the current study showed that there was a significant increase ($P < 0.05$) in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in the (T1) group as compared to (C) group, also, these results showed a significant ($p < 0.05$) decrease in the level of ALP, ALT, AST in the (T2, T3) groups compared to (T1) group. Several methods were used in the examination of the drug propylthiouracil nano-loaded on zinc oxide, as they included the following tests on the Fourier transform infrared Spectroscopy (FT – IR) and X-ray diffraction X-ray diffraction spectrum (XRD) as well as the use of atomic force microscope Atomic Force Microscope (AFM), the process of intercalation of the drug between layers of zinc oxide was carried out by the direct ion exchange method. This technique showed that propylthiouracil gave spectra indicating the success of the intercalation process, which was used in the subsequent stages of the study.
Accepted: June 04, 2023	
Published: June 20, 2023	

Keywords: Propylthiouracil, Omega-3, hyperthyroidism, thyroxine.



Introduction

Thyroid diseases resulting from a disorder in its functions, either hypothyroidism or hyperthyroidism, are one of the most common pathogens that are not determined by a specific age or gender, despite the disparity in the incidence rates between some communities, their disorder remains one of the most widespread health problems [1].

Hyperthyroidism is one of the diseases of the thyroid gland that causes an increase in its activity, as this increase is due to the high secretion of thyroid hormones T_4 and T_3 with a decrease in the secretion of TSH hormone and an increase in the secretion of the latter as a result of pituitary tumours [2].

Propylthiouracil is one of the most important drugs that have been used since the Forties of the last century in the treatment of hyperthyroidism, as it appeared on the world drug market at the beginning of 1940[3], which was widely used in the United States and Europe. The mechanism of action of the drug is based on inhibiting the synthesis of (T_4) and (T_3) hormone as well as the role of the drug affecting the hormones stored inside the thyroid gland and in the bloodstream [4].

Omega- 3 fatty acids, one of the most important dietary supplements, is characterized by its effectiveness in stimulating and producing many elements that maintain the balance of vital processes inside the body, as it is an important dietary supplement for infants and children, as well as its active role in the proper development of the brain during the embryonic period also the Omega-3 play an effective role in increasing the incidence of cardiovascular disease [5].

Researchers working in the field of practical applications of Nano pharmaceuticals are dealing with a high level of challenges that contributed to the development of research and studies in which this technology was invested to load drugs, which contributed to improving its therapeutic effectiveness while providing an accurate description of the safety of pharmaceutical nanomaterials with a low level of toxicity as a result of their high solubility and absorption, which reflected positively on their therapeutic efficiency by delivering the drug to the target tissues as well as reducing the side effects associated with the use of drugs Regularity [6].

Materials and Methods

Experimental animals

This study was conducted in the Animal House at the college of Science/University of AL-Qadisiyah, male albino rats were used, whose weight ranged between 150-250 grams and their age was between 3-4 months. The experimental animals were subjected to suitable laboratory conditions at a temperature of 20-25 °C, and the animals were provided with water and suspension during the duration of the experiment.

A-Experimental design

In this experiment, 28 male white rat animals were used, divided into four groups, each group included 7 animals. The study was designed as follows:

1-the first Group (C): included (7) animals, they were dosed with physiological saline solution NaCl at a concentration of 0.9% and returned as a control group.

2-the second Group (T1): included (7) animals in which thyroxine hyperthyroidism was developed at a concentration of 20 mg / kg of body weight by oral dosing using a special wine syringe for this purpose daily for 21 days.

3-the third group (T2): included (7) animals, in which thyroxine hyperthyroidism was developed at a concentration of 20 mg / kg body weight by oral dosing and then Omega-3 was dosed at a concentration of 100 mg / kg body weight using a special wine syringe for this purpose for 30 days.

4-the fourth group (T3) included (7) animals, in which thyroxine hyperthyroidism was developed at a concentration of 20 mg / kg of body weight by oral dosing and then propylthiouracil was dosed at a concentration of 15 mg / kg of body weight nano-loaded on zinc oxide using a special wine syringe for this purpose for a period of 30 days.

1-Measurements of Hormones and enzymes in the blood

The enzyme-Linked immunosorbent Assay (ELISA) immunoassay method described earlier was used to estimate the level of hormones in the serum and the absorbance was read at a wavelength of 450 nanometers (nm). We also use kits for each of the T₃, T₄ and TSH hormones produced by biocheck Inc. The British hormone insulin is produced by the American company Elabscience using the Elisa device.

The level of glutathione in the serum was estimated using the Elisa apparatus, and according to the kit manufactured by the company (SUNLONG).

The level of Malone dialdehyde in the serum was estimated using the Elisa apparatus, and according to the kit manufactured by the company (SUNLONG).

The concentration of SOD in the serum was estimated using the Elisa apparatus, and according to the kit manufactured by the company (SUNLONG).

The concentration of catalase in the serum was estimated using the Elisa device and according to the kit manufactured by the company (SUNLONG).

To estimate the effectiveness of aminotransferase enzymes Alto AST followed the colour method of the scientists Reitman and Frankel (1957) using kit-equipped assays from the Italian company Giesse.

Using the colour method followed [7], the efficacy of ALP was estimated by using kits prepared by the Italian Giesse company.

B-The development of hyperthyroidism

Levothyroxine country of origin, (Actavis, Bamstaple, USA) was used at a concentration of 20mg / kg body weight to induce hyperthyroidism[8], and after 21 days of dosing, samples were drawn from the blood of animals to confirm the occurrence of hyperthyroidism. Omega-3 country of origin, (AMS, USA) purchased from one of the pharmaceutical pharmacies in the city of Diwaniyah. The dose was used in the amount of 100 mg /kg of body weight of Omega-3 [9] and after dissolving the entire daily dose

of Omega-3 in oil[10], each animal was dosed daily with 1 ml orally using a special wine syringe for this purpose containing a hooked needle .

The propylthiouracil drug country of origin, (Quagen Pharmaceuticals, USA), which was obtained from one of the pharmaceutical pharmacies in the city of Diwaniyah. A dose of 15 mg/kg body weight of Propylthiouracil was used [11], and after dissolving the entire daily dose of Propylthiouracil in distilled water, each animal was dosed daily by 1 ml orally using a special wine syringe for this purpose containing a hooked needle.

The loading was carried out using the anti-hyperthyroidism drug (propylthiouracil) at a concentration of 20 mg /kg body weight [11], where it was nano-loaded on zinc oxide (USA) in the Common Diseases Laboratory / College of Veterinary Medicine / University of Al-Qadisiyah, after the drug (propylthiouracil) was ground using an electric grinder to obtain a fine powder and after successive steps the drug was loaded propylthiouracil on zinc oxide according to the method of [12].

C-Examination of the zinc oxide nano-loaded propylthiouracil drug:

Several methods were used in the examination of the drug propylthiouracil nano-loaded on zinc oxide, as they included the following tests on the Fourier transform infrared Spectroscopy (FT – IR) and X-ray diffraction X-ray diffraction spectrum (XRD) as well as the use of atomic force microscope Atomic Force Microscope (AFM) [13].

Blood Collection

After the end of the experiment, we used chloroform to anesthetize the animals and draw blood from the heart directly using the heart Puncture stab and by means of a medical syringe disposable syringe sterile capacity of 5 ml, and the drawn blood was placed in tubes containing EDTA where the blood was collected for the purpose of anticoagulant, left for 15- 20 minutes at Laboratory temperature, then the samples were placed inside the centrifuge type (Heraeus-Christ Gumby) at a speed of 3000 cycles/min for 15 minutes for the purpose of serum separation, serum isolation by micropipette micro mechanical pipette and placed in New plastic tubes for the purpose of conducting hormonal and biochemical tests, and the serum was kept at a temperature of - 20 M until use [14] .

Statistical analysis

The results of the experiments were analysed using the SPSS statistical program. The ANOVA test was used to compare the studied groups and the control group. The least significant difference (LSD) was calculated to test the significance of the results [15].

Results and Discussion

The level of thyroid hormones (T_4 , T_3) and hormone (TSH)

The results showed a significant increase ($P < 0.05$) in the level of thyroid hormones (T_4 , T_3) with a decrease in the level of TSH hormone in the hyperthyroid group (C) compared to the control group, and these results were consistent with the study [16,17]. This increase may explain the mechanism of action of L-Thyroxine, similar to the T_4 hormone, which is converted in tissues to T_3 by stimulating the enzymatic activity of target cells with accelerated use of ATP to supply the sodium - potassium pump with the necessary energy, which causes an increase in the secretion of thyroid hormones and thus hyperthyroidism developed in laboratory animals [18]. As for the decrease in the level of (TSH), it is explained by the effect of thyroxine, which is a substitute for the T_4 hormone, which turns into the T_3 hormone in the target tissues, as an increase in its concentration causes hyperthyroidism, which results in hormone-like antibodies that bind to thyroid-stimulating hormone receptors (TSHR), which is a protein receptor that contributes to the transmission of signals stimulating the production of thyroid hormones when bound to the TSH hormone, causing a constant urge thyroid hormones with a low level of Thyroid-Stimulating Hormone TSH, which is secreted by the negative feedback system between the level of thyroid hormones and the anterior pituitary from the other hand, and the hypothalamus on the other [19].

In contrast, an improvement in the level of thyroid hormones T_3 , T_4 in the blood serum was observed in the groups treated with Omega-3 and the drug nano-loaded on zinc oxide compared to the (C) Group, and this may explain the mechanism of action of propylthiouracil in inhibiting the biosynthesis of thyroxine inside the thyroid gland with inhibition of its peripheral transformation into T_3 in the bloodstream. The inhibitory function of the drug outside the thyroid gland is to block the formation (deiodinase type-1) by propylthiouracil through its interaction with selenenyl iodide for the synthesis of selenenyl sulphide inhibiting the process of transformation of T_4 to T_3 [20,21], may also explain the improvement in the aggregates that dosed the drug nano-loaded on zinc oxide to the size of the nanocarrier used to carry the drug due to the advantage of surface area versus volume and also the size affinity between nanoparticles and biomolecules inside the body, which facilitates the process of penetration of cellular membranes down to cellular organelles including the nucleus, which contributes to the regulation of intracellular reproduction and translation in target tissues [22].

The improvement in the level of TSH in comparison with C croupe is explained by the mechanism of action of the thyroid hormone regulatory drug, which prevents the binding of iodine to tyrosine by inhibiting the enzyme Peroxidase active in the oxidation of inorganic iodide (I^-) collected in thyroid cells to iodine (I) Iodine and, consequently, the high level of TSH by affecting the hypothalamic – pituitary - thyroid axis by negative feedback [23].

As for the improvement in the totals treated with Omega-3 compared to T1group, it is due to the content of omega-3 unsaturated fatty acids, which are known for their regulatory properties of various body functions, they are classified among the most

important anti-inflammatory and powerful oxidants that work to remove free radicals directly or indirectly by activating antioxidants such as GSH, CAT and SOD[24], where (EPA) is one of the most important fatty acids forming Omega-3, which makes up 18% of it, so it affects the level of thyroid hormones in two main directions, the first is the regulation of nerve signalling pathways by affecting the membrane lipids of thyroid cells, which contributes to increasing the sensitivity of TSH receptors as a result of the improvement in phospholipids of cell membranes on the one hand and the rapid and direct modification of the gene transcription process, which effectively contributes to reducing the level of T₃, T₄ hormones inside and outside the gland thyroiditis [25], and the improvement in the level of (TSH) is attributed to the effect of Omega-3 in enhancing the immune response and reducing inflammation, as it works to increase the stability of cell membranes and thus regulate the function of the immune system and prevent excessive inflammatory reactions, which is reflected positively on the regulation of the functions of the glands and the secretion of hormones, including the TSH hormone. Table (1).

Table (1): The effect of treatment with Omega -3 and nano-loaded propylthiouracil at the level of (T₄, T₃, TSH) in male white rats

Groups Parameters	C	T1	T2	T3	LSD
T4	46.9±4.12 d	73.5 ± 10.2 a	64.8±4.43 b	56.3±3.68 c	5.23
T3	1.73±0.04 d	2.81 ± 0.08 a	2.51 ±0.09 b	2.13±0.11 c	0.21 1
TSH	0.537±0.25 a	0.237±0.003 d	0.328±0.03 c	0.44 ±0.03 b	0.03 4

The numbers represent the mean ± standard error. The different letters indicate that there are significant differences (P<0.05) between the groups.

The level of antioxidant

The results showed a significant decrease (0.05>P) in the level of antioxidants (CAT, SOD, GSH) with an increase in the level of (MDA) in the group in which thyroxine hyperthyroidism was developed for 21 days compared to the control group, and these results were consistent with the study [16,26]. This may be explained by hyperthyroidism, which contributed to the increase in metabolism by increasing the speed of the basal metabolic rate in the body by stimulating cellular oxidation through high consumption of mitochondrial oxygen with increased expression of Na⁺ / K⁺ATPase, an enzyme that carries out the active transport of potassium and sodium in opposite directions, as it derives the necessary energy for this from the decomposition of ATP molecules [27], high rates of carbohydrate and lipid metabolism as well as the destruction of proteins, including carbonyl protein, a protein produced from the oxidation of proteins inside cells as a result of damage to enzymes by oxidative stress, which is associated with high oxygen consumption, which constitutes a risk factor resulting in

increased oxidation inside cells in conjunction with the high level of free radicals, which causes oxidation of many biomolecules as well as DNA damage and lipid peroxidation resulting from an imbalance between oxidants and antioxidants, as the high level of oxidants is accompanied by a decrease in antioxidants it is a state of oxidative stress that results in an imbalance between ROS and the body's ability Bioassay on the detoxification of reactive intermediates that result in damage to organ tissues [28]. On the other hand, the results showed a significant increase in the level of antioxidants SOD, GSH and CAT accompanied by a decrease in the level of MDA in the aggregates that were treated with free and nano-drug, and this improvement may be explained by the role of free and nano-drug in regulating the level of thyroid hormones, which contributed to regulating the level of hydroxyl radical (OH⁻) and hydrogen peroxide H₂O₂ and superoxide ion (O₂⁻) in the liver, causing a balance of cellular and enzymatic oxidation and reduction processes with a high level of antioxidants, thereby reducing therefore, the decrease in the level of SOD, GSH and CAT in the totals treated with thyroxine is attributed to their role in preventing oxidation The study of Bednarek and his group (2004) confirmed that the treatment of patients with toxic multinodular Goiter and Graves' disease with propylthiouracil contributed to reducing the level of hydroxyl radical, hydrogen peroxide and superoxide ion and then scavenging free radicals, while confirming that the treatment of patients with toxic multinodular Goiter and Graves' disease with propylthiouracil contributed to reducing the level of hydroxyl radical, hydrogen peroxide and superoxide ion and the study of [29] that the dosing of mice with thyroxine at 0.1 mg /kg body weight contributed to an increase in the level of the radical H₂O₂ and Ion (O₂⁻), also explains the improvement in The groups that dosed Omega-3 to the role of long-chain unsaturated fatty acids represented by EPA, ALA and DHA in promoting the synthesis of antioxidants, including SOD, which converts the superoxide negative superoxide radical to hydrogen peroxide and then converts the cat radical H₂O₂ to water and oxygen, while GSH is inside the cells either in the form of oxidized disulfide (GSSG) or in the case of reduced glutathione, as its level is inversely correlated with the state of oxidative stress [30], a series of studies conducted on women with breast cancer confirmed that the intake of Omega-3 acids before and after chemotherapy contributed to an increase in their antioxidants, which enhanced the body's defence capabilities [31].Table (2) .

Table (2): Effect of treatment with Omega -3 and nano-loaded propylthiouracil at the level of (GSH, SOD, CAT, MDA) in male white rats.

Groups Parameters	C	T1	T2	T3	LDS
GSH	345±193.4 a	235.2±159.6 d	267.2±231.7 c	288.1±41.04 c	29.51
SOD	62.208±30.07 2	21.899±80.2 23	33.882±90.0 42	37.948± 30.326	7.271

	a	d	c	c	
CAT	23.04±1.94	14.7±0.74	18.37±0.65	18.42±0.52	
	a	d	c	c	1.78
MDA	101.9±7.05	155.3±41.1	141.2±9.58	125.5±6.7	
	e	a	b	c	6.7

The numbers represent the mean \pm standard error. The different letters indicate that there are significant differences ($P < 0.05$) between the groups.

The level of liver enzymes

The results showed a significant increase ($P < 0.05$) in the level of liver enzymes (AST, ALT, ALP) in the group in which thyroxine hyperthyroidism was developed for 21 days compared with the control group, and these results agreed with the study [32], which confirmed elevated liver enzymes after treatment of animals with thyroxine to induce hyperthyroidism 12 days after daily dosing. Since the reason for the high level of liver enzymes (AST, ALT, ALP) is associated with an increase in lipid peroxidation in organs, and because the liver is one of the tissues rich in mitochondria, so it is more susceptible to oxidative damage that affects the function of mitochondria, which weakens the effectiveness of the electron transport chain, which leads to programmed cell death [33], confirmed that the high level of aminotransferase enzymes as a result of damage in the liver, which is negatively reflected on the activity of the liver, resulting from oxidative stress and the formation of free radicals, especially they are an indicator of pathologies of some organs of the body, especially the liver [27].

On the other hand, the current results showed a significant decrease ($P < 0.05$) in the level of liver enzymes for the groups treated with nano-loaded drug and Omega -3 compared to the hyperthyroid group, Liver enzymes have improved markedly, which indicates a clear improvement in overall health and the integrity of the liver and other organs, and the study found that the use of propylthiouracil at a dose of 10 mg /kg and for 12 days reduced the levels of ALP, ALT, AST in male rats treated with thyroxine for 12 days [32], this may explain the role of the drug in inhibiting the enzyme deiodinase enzymes (DIO) it is expressed in the liver, kidneys and thyroid follicles, which contributes to inhibiting the production of T_3 in the bloodstream and thus reducing the high levels of thyroid hormones, as dysfunction in the thyroid gland coincides with a disorder in liver function, where Thyrotoxicosis coincides with the constant stimulation of the thyroid gland to produce (T_4 and T_3) hormones, which is reflected by damage to the liver, causing a state of dysfunction and then programmed death of hepatocytes [33], as well as the role of nanocarriers in enhancing the therapeutic index of the drug and in a study [34] confirmed that nanoparticles have a therapeutic effect that targets liver diseases such as malignant tumor or hepatitis, as the liver tissue and its cells have the ability to detain these particles and then benefit from the drug loaded on the nanoparticles for a longer period, and this may be one of the most important reasons that explain the improvement in the Omega-3-dosed aggregates is due to its rich content of

unsaturated fatty acids, which contribute to the protection of liver tissue from damage by inhibiting the heptanuclear factor nuclear factor Kappa B (NF-kB). Table (3).

Table (3): Effect of treatment with Omega -3 and nano-loaded propylthiouracil at the level of (AST, ALT, ALP) in male white rats.

Groups Parameters	C-	T1	T2	T3	LDS
ALP	95.52±30.3 d	155.2±69.3 a	128.7±31. 4 b	108.1±18.6 c	5.9
ALT	23.25±17.3 d	48.84±30.1 a	39.22± 6.32 b	30.3±14.1 c	4.58
AST	40.7±19.2 d	65.4±35.6 a	57.2±5.18 b	48.5±29.7 c	4.73

The numbers represent the mean ± standard error. The different letters indicate that there are significant differences (P<0.05) between the groups.

Nanoparticle Assay of Propylthiouracil zinc oxide nanoparticle assay

In this study, a number of tests were used to confirm the loading of propylthiouracil on zinc oxide nanoparticles, which included the following

Fourier-Transform Infrared (FTIR) Spectroscopy infrared spectrometer

The drug propylthiouracil at a concentration of (15 mg/kg) of body weight was used in the treatment of hyperthyroidism in this study to manufacture a drug nano-loaded on zinc oxide, as the intercalation of the drug between zinc oxide layers was carried out by direct ion exchange. This technique showed that propylthiouracil gave spectra indicating the success of the intercalation process, which was used in the subsequent stages of the study Figure (1,2).

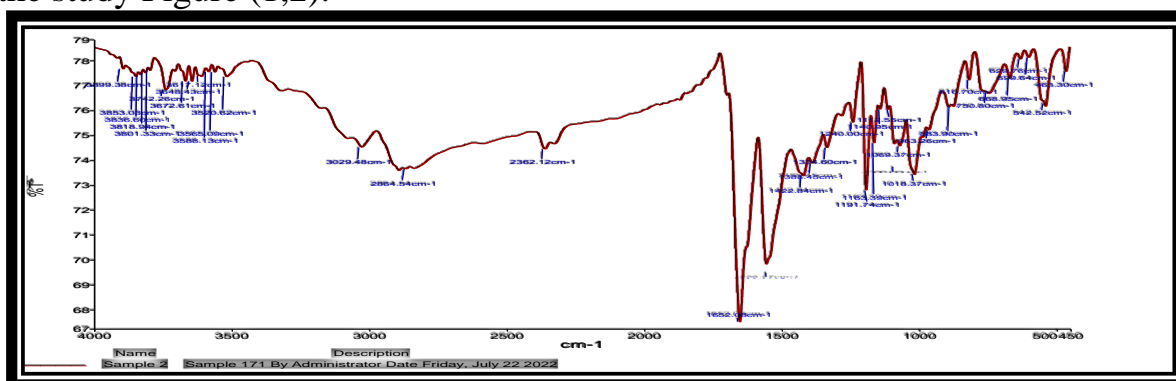


Figure (1): shows the infrared spectrometer images of the drug propylthiouracil

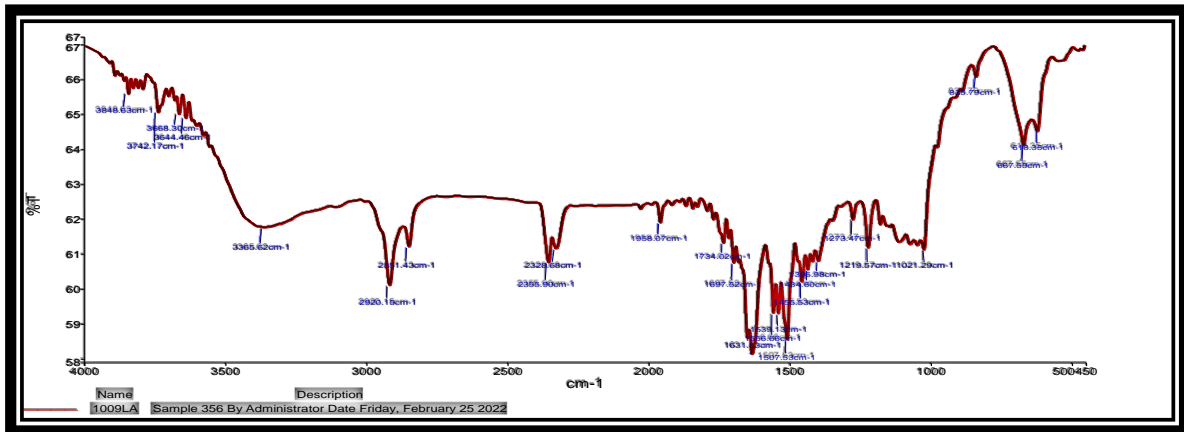


Figure (2): shows the infrared spectrometer images of the ZnO surface loaded with the drug propylthiouracil

X-ray diffraction X-Ray Diffraction (XRD)

The surface of ZnO particles loaded with the drug in its solid state was tested using X-ray diffraction, to find out some of its structural properties such as crystal phase, crystal size and its purity can also be estimated. The figure (2) shows the X-ray diffraction spectrum of ZnO particles loaded with the drug. The Debye-Scherrer-Debye-Scherrer equation was used to calculate the crystal size of ZnO nanoparticles and the drug as follows [35] Figure (3,4).

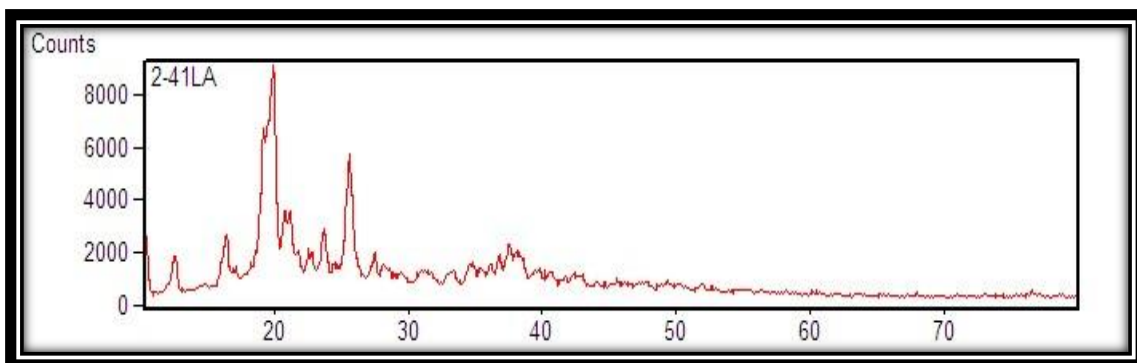


Figure (3): x-ray diffraction of the ZnO surface

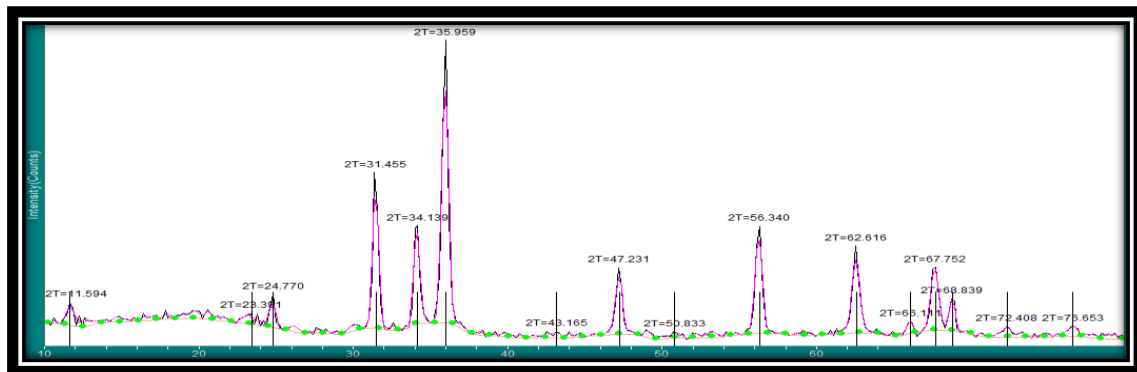


Figure (4): x-ray diffraction of the ZnO surface loaded with the drug

Atomic force microscope (AFM) Atomic Force Microscope

Through the use of an atomic force microscope, statistical information is obtained about the external topography of the surface of the prepared gel overlay, and the values of the extent of distribution, homogeneity, thickness and roughness of the Surface Roughness depending on the square root of the mean roughness root Mean Square Roughness(RMS) [6], which is observed by Figure (5,6), which shows the three-dimensional image of the surface loaded with the drug with the indication of the most important statistical values of surface roughness.

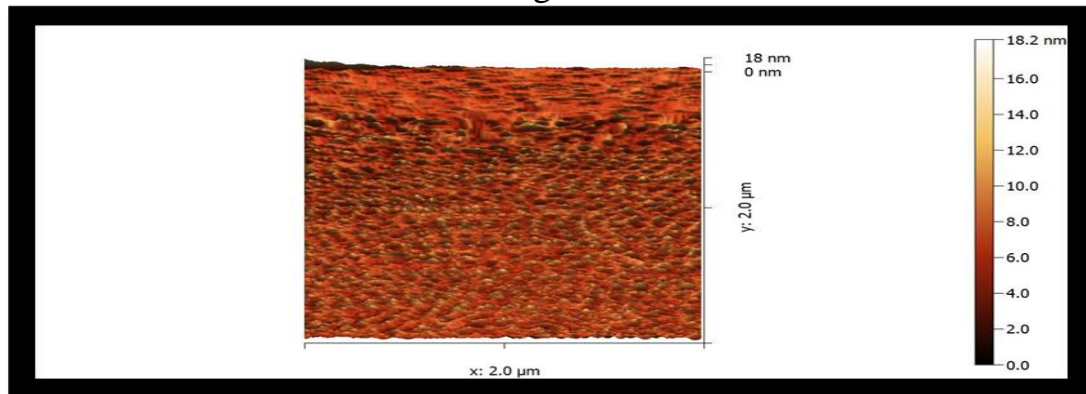


Figure (5): shows the drug propylthiouracil with dimensions (18nm*y:2.0 μm*x2.0 μm)

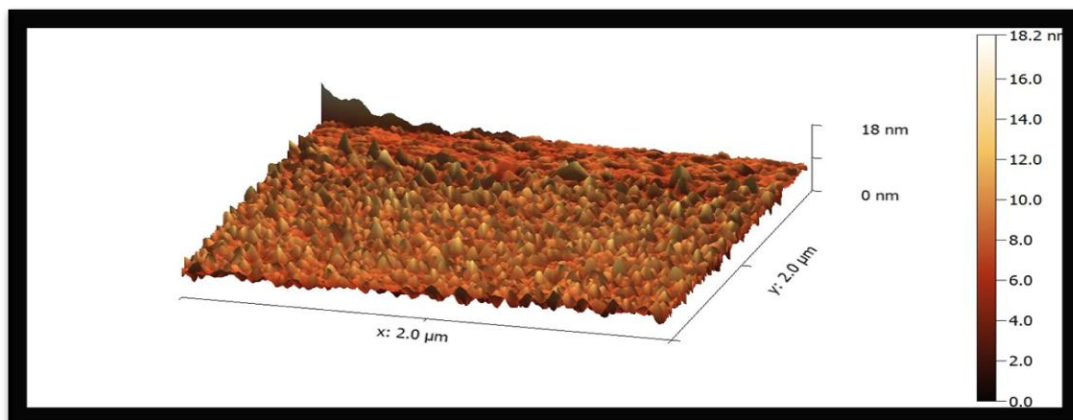


Figure (6): shows the drug loaded on the surface of ZnO with dimensions (18nm*y:2.0 μm*x2.0 μm)

Scanning electron Microscope (SEM)

The properties of the surface morphology of ZnO particles loaded with the drug were studied in terms of the size and shape of the particles and aggregations among them aggregations, in addition to the distribution of these particles using the scanning electron microscope technique. As shown in Figure (7).

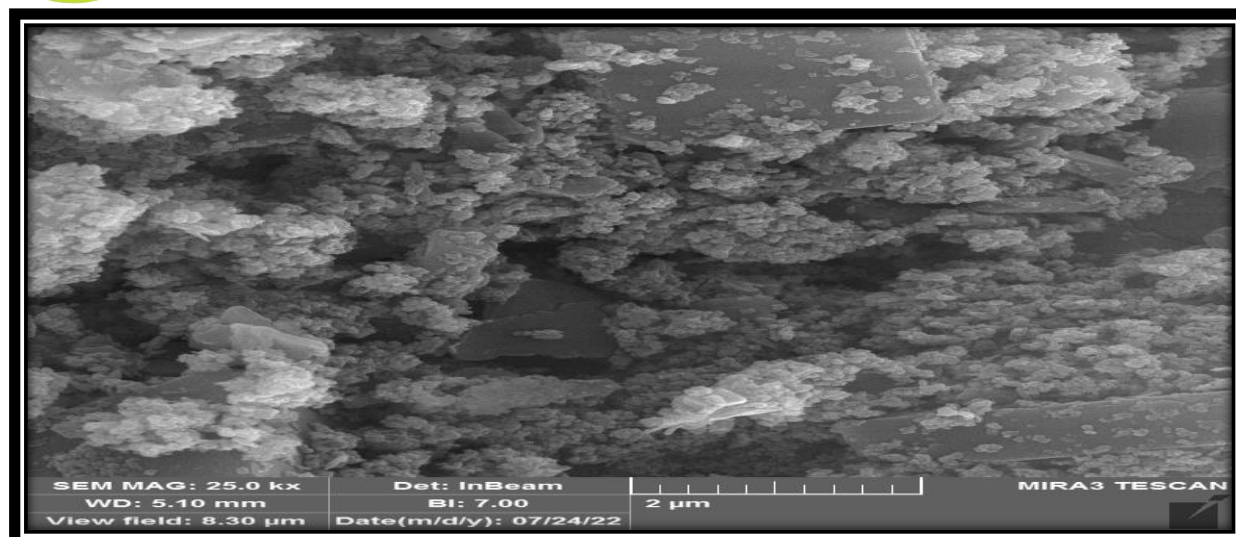


Figure (7): The electron microscope (SEM) of the drug propylthiouracil nano-loaded on zinc oxide

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