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The antioxidant Effect of Allicin Nanoparticles in Some Physiological Parameters in Hyperthyroidism Experimental Induced Female Rats

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Abstract

The present study has been designed to investigate impact of allicin nano particles and methimazole on female rats with induction of the hyperthyroidism. The study has been conducted on adult female rats at the department of physiology and pharmacology, College of Veterinary Medicine, Al-Qadisiya University during the period extended from October 2022 and Feb. 2023. Fifty mature female rats (aged 90 days and weighted 150±10 g) have been randomly assigned to 5 equal groups (10 each), C: group was drenched orally with1 ml drinking water as control group T1,T2,T3 and T4 groups were drenched orally with thyroxine (300mg/kg of B.W dissolved in 1 ml distilled water for 28 days) for hyperthyroidism induction, T2 group was drenched orally with methimazole (2.5 mg/kg of B.W dissolved in 1 ml distilled water for 30 days), T3 group was drenched orally with allicin nano particles (50 mg/kg of B.W dissolved in 1 ml distilled water for 30 days) and T4 group was drenched orally with methimazole and allicin nanoparticles for 30 days. At the end of experiment the animals were sacrificed and blood samples were taken from the heart, serum samples were separated from blood for assessment of Antioxidants (SOD, GSH, CAT and MDA) and Lipid profile (Cholesterol, Triglyceride, HDL and LDL). The results showed a significant difference ($p<0.05$) in MDA level between experimental groups increasing in T1 group as compared with control group while decreasing in T2,T3 and T4 groups as compared with T1.The results illustrated a significant difference ($p<0.05$) in body weight and antioxidants(SOD,GSH and CAT) between experimental groups represented by decreasing these parameters in T1 group as compared with control group, while increasing in T2,T3 and T4 groups as compared with T1. The levels of HDL, Cholesterol and Triglyceride decreased in T1 group as compared with control group while increased in T2,T3 and T4 groups as compared withT1 group. LDL level indicated a significant increase($p<0.05$) in T1 group as compared with control group while decreased in T2,T3 and T4 groups as compared with T1 group.

Keywords: Allicin nanoparticles, Methimazole, hyperthyroidism, Antioxidants, Lipid profile.

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التأثير المضاد للأكسدة لجزيئات الأ لسين النانوية في بعض المعايير الفسلجية لإناث الجرذان المستحدث فيها فرط الدرقية تجريبيا

فرع الفسلجة والادوية، كلية الطب البيطري، جامعة القادسية، العراق
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الخلاصة

تم تصميم الدراسة الحالية للتحري عن تأثير جسيمات الأليسين النانوية و عقار الميثيمازول على إناث الجرذان المستحدث فيها فرط نشاط الغدة الدرقية. تم إجراء الدراسة على إناث الجرذان البالغة في فرع الفسلجة والادوية/ كلية الطب البيطري / جامعة القادسية خلال الفترة الممتدة من تشرين الاول 2022 الى شباط 2023. تم اخذ خمسين حيوان (بعمر 90 يومًا ووزن 150 ± 10 جم) وتقسيمها عشوائيًا الى خمسة مجموعات متساوية (10 حيوان في كل مجموعة). المجموعة C : جرعت بماء الشرب كمجموعة سيطرة، المجموعات T1 و T2 و T3 و T4تم تجريعها عن طريق الفم بالثيروكسين (300 ملغم/كغم من وزن الجسم مذابة في 1 مل من الماء المقطر لمدة 28 يومًا) لتحفيز فرط نشاط الغدة الدرقية. المجموعة T2 جرعت عن طريق الفم بالميثيمازول (2.5 ملغم/كغم من وزن الجسم مذابة في 1 مل من الماء المقطر لمدة 30 يومًا). المجموعة T3جرعت عن طريق الفم بجزيئات الأليسين النانوية (50 ملغم/كغم من وزن الجسم مذابة في 1 مل من الماء المقطر لمدة 30 يومًا). المجموعة T4 جرعت عن طريق الفم بالميثيمازول وجزيئات الأليسين النانوية لمدة 30 يومًا. في نهاية التجربة، تم التضحية بالحيوانات وأخذ عينات الدم من القلب، وتم فصل عينات السيروم من الدم لتقييم مستويات مضادات الأكسدة (SOD و GSH و CAT) و MDA وصورة الدهون (الكولسترول والدهون الثلاثية و HDL و LDL). أظهرت النتائج وجود فرق معنوي ($P<0.05$) في مستوى MDA بين المجموعات التجريبية حيث زاد في المجموعة T1 مقارنة مع مجموعة السيطرة بينما انخفض في المجموعات T2 و T3و T4مقارنة مع T1 . وأظهرت النتائج وجود فرق معنوي ($P<0.05$)في وزن الجسم ومضادات الأكسدة (SOD,GSH,CAT) بين المجموعات التجريبية ممثلة بانخفاض هذه المعايير في مجموعة T1 مقارنة مع مجموعة السيطرة، في حين ارتفعت في مجموعات T2,T3و T4مقارنة مع T1 انخفضت مستويات HDL والكوليسترول والدهون الثلاثية في مجموعة T1 مقارنة بمجموعة السيطرة بينما ارتفعت في مجموعات T2 و T3و T4مقارنة بالمجموعة .T1 أشار مستوى LDLإلى ارتفاع معنوي ($p<0.05$) في المجموعة T1 مقارنة بمجموعة السيطرة بينما انخفض في المجموعاتT2 ، T3و T4مقارنة بالمجموعةT1.

الكلمات المفتاحية: جزيئات الأليسين النانوية، الميثيمازول، فرط نشاط الغدة الدرقية، مضادات الأكسدة، صورة الدهون.

Introduction

The thyroid gland produces, stores and secretes T4 via a negative feedback system involving the hypothalamus and pituitary gland. Thyroid dysfunction happens when any one of the following high or low levels of free thyroid hormones (THS) thyroid stimulating hormones (TSH). indicate that this mechanism is disturbed thyroid disorders can have many different possible causes, including autoimmune diseases like Grave's disease, infections like post viral inflammation, other systemic medical conditions, medications, like lithium and amiodarone, nutritional excess or deficiencies like iodine, tumors of the thyroid or less frequently, the pituitary trauma, or pregnancy (1). New pharmacologically active compounds have been discovered by using medicinal plants as a source. Natural products, for

instance, could be a suitable substitute for managing the pathogen linked to disorders (2,3). One of the oldest and most significant herbs that have been utilized as traditional medicine since ancient time is garlic (*Allium sativum*) an aromatic annual spice (4,5). lt is regarded as the second most widely used species of Allium after anion (*Allium cepa*), which is used as a treatment for a number of common ailments such the common cold, the flu, snake bites, and hypertension (6), According to studies, allium species and their active ingredients lower the risk of diabetes and heart disease, defend against infections by boosting the immune system, and have antibacterial, antifungal and anti-aging properties anti-cancer and anti-aging qualities that have been verified by epidemiological data from human cllinical investigation (7). Garlic has been shown in studies to protect a number of bodily systems. This investigation looked at how garlic affected serum levels of T3 and T4 being given rats. Rats receiving garlic extract had serum levels of T3 and T4 that were not substantially different from the rats in the control group. The research indicates that garlic extract has inhibitory effects against elevated thyroid function in rats. (8). According to studies, traditional medicine can influence the anterior pituitary's ability to release hormones impair the thyroid gland's functionality (9). Which in turn may affect the function and metabolism of the testes. The hypothalamic - pituitary- thyroid (HPT) axis appears to be more resistant to the psychost imulant action than other data suggest. (10). Therefore, the purpose of this study was to assess any potential synergistic effects that these extract may have on thyroid hormones and other physiological indicators. which hyperthyroidism influences. (11). In hyperthyroid rats, serum levels of triiodothyronine (T3), thyroxine (T4), TG, significantly while TSH, LDL, HDL, LDL/HDL, and TC, total protein, and percentage of body fat significantly decreased change in weight relative. Most of the examined parameters improved after oral administration of these extracts. Abnormalities in physiology and /or medicine are caused by changes in hormone levels, particularly thyroid hormone changes (12). Allicin has signifiantly greater bioactivity than other chemical constituents of garlic, particularly antioxidant activity (13).

Materials and Methods

Experimental animals:

The study has been conducted on adult female rats at the department of physiology and pharmacology, College of Veterinary Medicine, Al-Qadisiya University during the period extended from October 2022 and Feb. 2023. Forty mature female rats (aged 90 days and weighted 150±10 g) . Fifty adult female Wister rats were divided randomly into five equal groups: The first group(C) was given 1ml of distilled water daily for 30 days considers as positive control group. The second(T1), third(T2), fourth(T3) and fifth(T5) groups were given L-thyroxine

orally at dose 300 mg/kg of body weight dissolved in 1 ml distilled water once daily for 28 days for hyperthyroidism induction. (14). T1 group considers as negative control, T2 was given methimazole orally at dose 2.5mg/kg of body weight(2.5 mg/kg of B.W dissolved in 1 ml distilled water once daily for 30 days. T3 was given allicin nano-particles orally at dose 50 mg/kg of body weight dissolved in 1 ml distilled water once daily for 30 days (15). T4 group was given methimazole and allicin nano-particles once daily for 30 days (16).

Female rats were anesthetized (0.3 ml ketamine + 0.1 ml xylazine/ kg b.w. ip), dissected, and blood samples were taken from the heart using non-heparinized tubes. Separated serum from blood was centrifuged at 3000 rpm for 5 minutes and stored at -20 degrees Celsius until used for measurement of antioxidant (SOD, GSH, CAT and MDA), and Lipid profile (Cholesterol, Triglyceride, HDL and LDL).

Preparation of Nano composites:

50 g of allicin was dissolved in BBS phosphate solution,and the material was placed in the Vortex Mixer for five minutes, and then it was placed in the intellingnt ultrasonic processor using a device of UP200ht Hielscher at the internal medicine Laboratory/Al-Qadisiyah University,College of Veterinary Medicine at a temperature of 50 watts period of 30 miuntes, a solution was obtained research to convert the solution in to Nana size. After that stored in 4oC until use in which allicin particle size reached 60nm (17)

Laboratry measurements:

1-Body weight was estimated by using a specialized scale .

2- Antioxidant status (MDA, SOD,GSH and CAT)were estimated by using kits from antioxidants Company ،BioSolar Country China.

3- Lipids profiles (HLD, Cholesterol, Triglyceride and LDL)were estimated by using kits from Company Biosystem , Country USA .

Ethical approval

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

Statistical Analysis: The data was summarized using a mean ± standard deviation format (SEM). One-way analysis of variance (ANOVA) with Least Significant Difference (LSD) were used to compare the means of the different groups the cutoff for significance was set at ($P<0.05$). All analyses were statistically analyzed using (SPSS Institute, Inc., USA) (18).

Results

Characterization of Nano-particles:

1-UV- Visible Spectrophotometer

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study. The wave length of nanoparticles was measured from 220 to 1100 nm for wave length by recording UV-visible spectrum of standard solution. Maximum absorbance (λ max0.403) was shown at 301nm (figure 1)

Fig (1) UV- Visible spectrophotometer of Allicin nanoparticles

2- SEM analysis

The morphology of nanoparticles was also confirmed in terms of nano size utilizing scanning electron microscopy (SEM). The SEM images shown in fig. (2) exhibited those particles have irregular shape with a smooth exterior. The average diameter of The obtained NPs evaluated by SEM showed diameter range from 54 to 84 nm.

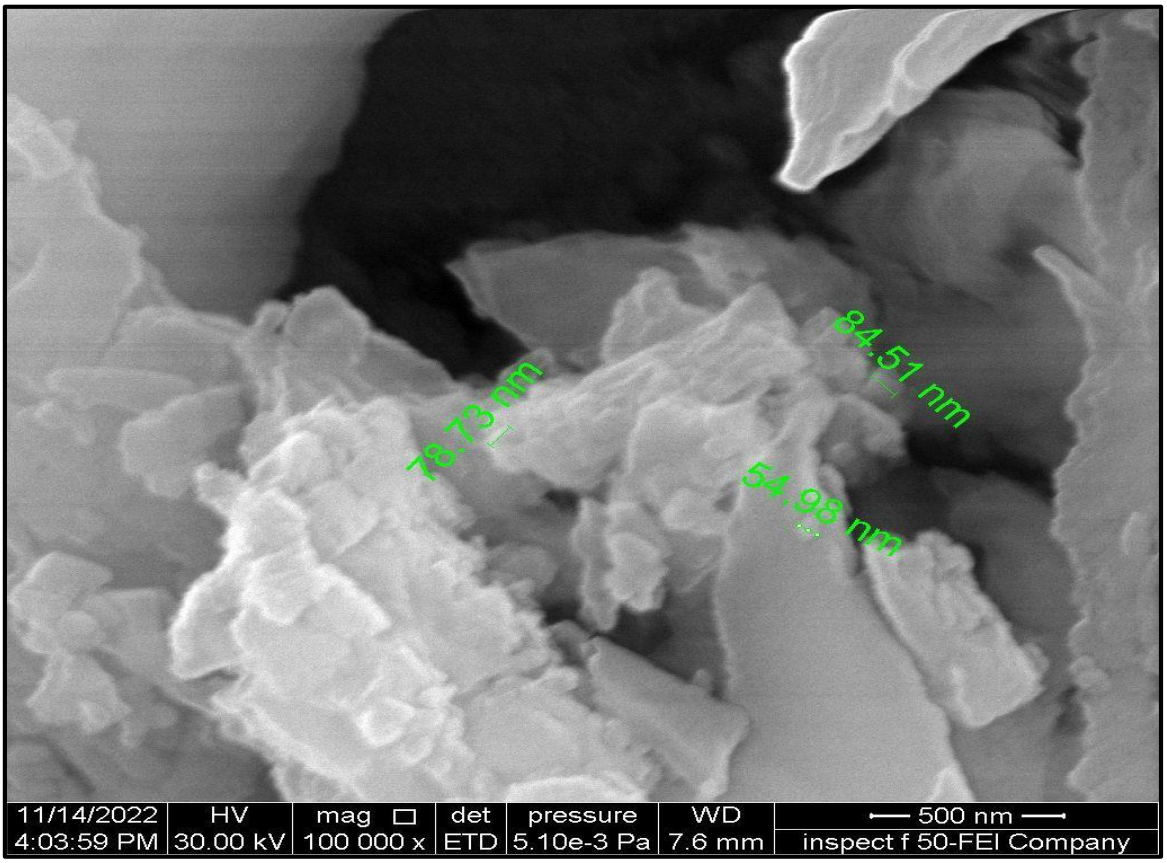

Fig 2: SEM of nanoparticles of appear elongated to irregular shape of Allicin nanoparticles with particle size around 500 nm.

3- FTIR Spectroscopy

FTIR spectroscopy of exhibited the presence of bending vibration of the aliphatic CH bond at the peaks 2079.45 cm -1. As for the peaks at numbers 1636.39 cm-1 , they may be due to the presence of a vibrational bending of C=O and C-O, due to the aromatic amine group, respectively. AsOH was found at the wave numbers 3859.15 and 3436.13cm-1 , was attributed to the carboxylic groups. The FTIR spectra for showed appear new functional groups, utilized to predict the chemical and biological activities of biomolecules. Following descriptions are FTIR results that could be interpreted for effective use of the plant products. The presence of esters (S-OR) group at 687.58 cm-1.

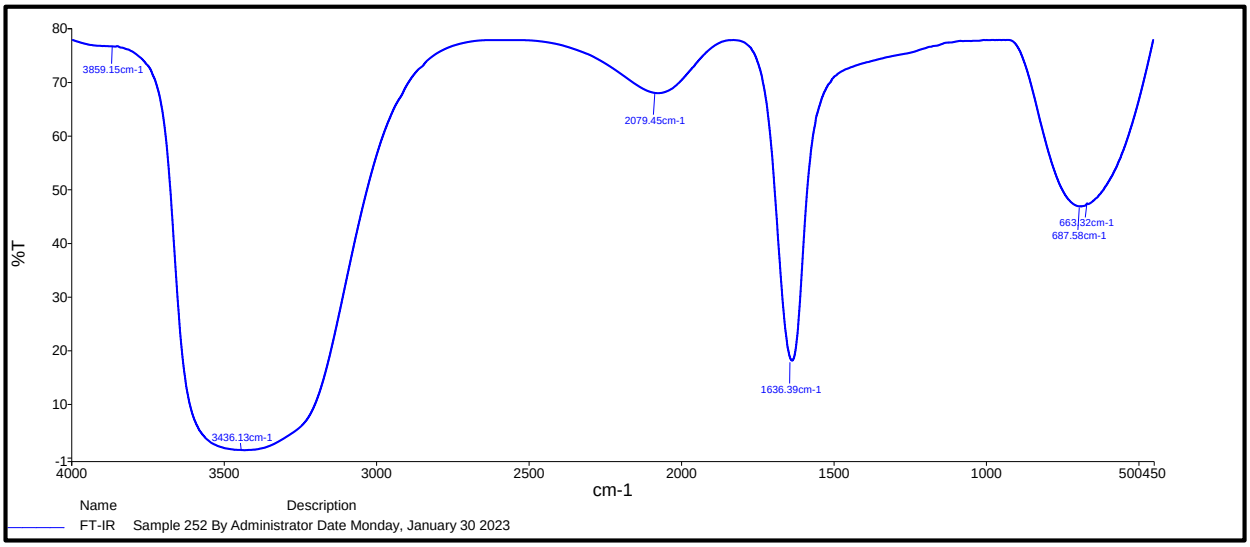

Figure 3 showed that FTIR spectra of paul Scherer equation used to determination of size crystals in the form of powder

4- X-ray diffraction

The Scherer equation can be written as: Dp = (0.94 X λ) / (β X Cosθ), Where, Dp = Average Crystallite size, β = Line broadening in radians, θ = Bragg angle, λ = X-Ray wavelength. The present data of XRD allicin recorded four main peak 2Theta at 15.212, 33.346, 47.308 and 77.99 with their FWHM 0.666, 0.619, 0.549 and 0.848to recorded size at 13,14,17 and 13 nm respectively figure(a). In common, the material in crystalline state appear a series of sharp peaks, while amorphous materials produce broad and non-defined peaks Fig. 4 showed a very broad peak,

indicating that the bilayer showed 2-3 strong peaks (Fig. 4). The occurrence of the second peak indicative of more structured systems, suggests that the extracts are interposed between the chains of phospholipid bilayer and gives a better flow ability. XRD analysis revealed the crystalline nature of the extracts within the which indicated better solubility in aqueous medium.

Fig 4 Shows a very broad peak

Body weight

The results illustrated in table1 showed a significant differences ($p<0.05$) in body weight between experimental groups represented by decreasing in the body weight in T1 group (125.12 ± 0.492 gm.) as compared to the control group (184.87 ±.319 gm.). Whereas the results indicated there was a significant increase in body weight in T2, T3 and T4 groups (193.37 ± 0.771, 136.37 ± 1.077 &165.87 ± 0.673) gm. respectively as compared with T1 group. T2 group recorded a highest value than T3 and T4 groups.

Table 1: Effect of Thyroxine, Methimazole and Allicin Nanoparticles on Body Weight (gm) in Mature Female Rats

Groups	Mean ± SEM
C	184.87 ± 0.319^{B}
T1	125.12 ± 0.492^{E}
T2	193.37 ± 0.771^{A}
T3	136.37 ± 1.077^{D}
T4	165.87 ± 0.673^{C}
LSD	5.214

The results represented as mean ± SE. Different latters denote the presence of significant differences ($P<0.05$) between groups.
C: female rats drenched orally with drinking water (1 ml).
T1: female rats drenched orally with thyroxine (300 mg/kg of B.W suspended in 1 ml of drinking water) for 28 days.
T2: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.
T3: female rats drenched orally with thyroxine and treated with allicin nano particles (50 mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.
T4: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W) and allicin nanoparticles (50 mg/ kg of B.W) for 30 days.

Antioxidants Status:

The results illustrated in table 2 showed a significant differences ($p<0.05$) in MDA represented by increasing it in T1 group (5.375 ± 0.0879) that was dosed with thyroxine compared with the control group (1.601 ± 0.0036).In contrast to the T1 group the methimazol-treated T2 group demonstrated a statistically significant reduction (1.851 ± 0.0104).In comparison to T1 and T2,the present study found a notable reduction in the allicin nanoparticles dosed T3 group(0.895 ± 0.0113). Additionally ,in comparison to T2,T3, the current study found a substantial rise at T4(2.306 ± 0.0302) when treated with a mixture of methimazole and allicin nanoparticles. Table 2 display the results of the current study , which demonstrated that there were significant differences($p<0.05$) in the levels of SOD, GSH, and CAT between the experimental groups. Specifically, when thyroxin was administrated to the T1 group, there was a significant decrease ($p<0.05$) in these three parameters(1.21 ± 0.0094 ,1.33 ± 0.0062, and 0.32 ± 0.0029) respectively compared to the control group (2.17 ± 0.0041,2.51 ± 0.0076, and 0.63 ± 0.0042)respectively. The current study's results demonstrated a significant increase ($p<0.05$) in the methimazole-dosed T2 group(2.028 ± 0.0446, 2.102 ± 0.0823, and 0.44 ± 0.0158) respectively as compared to the T1 group. Compared to the T1 and T2,the current study found a significant increase in T3 group(5.165 ± 0.0875, 6.36 ± 0.0749, and 0.90 ± 0.0120)respectively when allicin nanoparticles administrated.Similarly,T4(2.94 ± 0.0227, 3.99 ± 0.0323, and 0.67 ± 0.0082) respectively when a combination of methimazole and allicin nanoparticles were administrated showed a significant increase in comparison to T1and T2.

Table 2: Effect of Thyroxine, Methimazole and Allicin Nanoparticles on Antioxidants in Mature Female Rats

Groups	MDA (µmole/ml)	SOD (U/ml)	GSH (µMole/ml)	CAT (U/ml)
C	1.601 ± 0.0036^{D}	2.17 ± 0.0041^{C}	2.51 ± 0.0076^{C}	0.63 ± 0.0042^{C}
T1	5.375 ± 0.0879^{A}	1.21 ± 0.0094^{E}	1.33 ± 0.0062^{E}	0.32 ± 0.0029^{E}
T2	1.851 ± 0.0104^{C}	2.028 ± 0.0446^{D}	2.102 ± 0.0823^{D}	0.44 ± 0.0158^{D}
T3	0.895 ± 0.0113^{E}	5.165 ± 0.0875^{A}	6.36 ± 0.0749^{A}	0.90 ± 0.0120^{A}
T4	2.306 ± 0.0302^{B}	2.94 ± 0.0227^{B}	3.99 ± 0.0323^{B}	0.67 ± 0.0082^{B}
LSD	0.182	0.453	0.685	0.021

The results represented as mean ± SEM.

Different latters denote the presence of significant differences ($P<0.05$) between groups.

C: female rats drenched orally with drinking water (1 ml).

T1: female rats drenched orally with thyroxine (300 mg/kg of B.W suspended in 1 ml of drinking water) for 28 days.

T2: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.
T3: female rats drenched orally with thyroxine and treated with allicin nano particles (50mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.
T4: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W) and allicin nanoparticles(50 mg /kg of B.W)for 30 days.

Lipids profiles:

There was a significant difference ($p<0.05$) in HDL levels of the experimental groups, as shown in table 3 in this study, the thyroxin dosed T1 group had a significant lower level(32.62 ± 0.611) as compared with the control group (53.37 ± 0.631).on the other hand, the methimazole-dosed T2 group had a significantly higher level (44.62 ± 0.498) than the T1 group. The present study's findings also revealed a notable reduction at T3 group (64.75 ± 0.494) in coparison to the T1 and T2. The results showed a significant decrease in HDL in T4 group (37.87 ± 0.413) in comparison to T2 and T3 groups. While HDL inT4 group was higher than T1group. The results illustrated in table 3 indicated a significant decrease ($p<0.05$) in Cholesterol and Triglyceride levels in T1 group(81.75 ± 0.542 and 118.75 ± 0.932) that was received thyroxine compared with the control group(108.87 ± 3.316 and 151.12 ± 0.401).On the other hand, T2 group that received methimazole showed a significantly higher Cholesterol and Triglyceride levels(91.62 ± 0.546 and 122.75 ± 0.440) in comparison to the T1 group. The results of the current study showed a significant decrease ($P<0.05$) in T3 group (53.62 ± 0.865 and 91.62 ± 0.961) which was dosed with allicin nanoparticles compared to the T1 and T2 groups, also decrease in T4 group (67.37 ± 0.467 and 101.75 ± 0.767) which was dosed with mix methimazole and allicin nanoparticles compared with the T1and T2 groups .While T4 group was higher than T3 group. The results illustrated in table 3 showed significant differences ($p<0.05$) between experimental groups in LDL level, which increased in T1 group (123.75 ± 0.494) compared with the control group(147.62 ± 0.473) , While decreased in T2 group (102.75 ± 1.116) compared with the T1group, also increased in T3 group (148.125 ± 0.843) compared to the T1 and T2 groups, also increased in T4 compared with the T1,T2and T3 groups.

Table 3: Effect of Thyroxine, Methimazole and Allicin Nanoparticles on lipids profiles in Mature Female Rats

Groups	HDL (mg/dL)	Cholesterol. (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)

C	53.37 ± 0.631^{B}	108.87 ± 3.316^{A}	151.12 ± 0.401^{A}	123.75 ± 0.494^{D}
T1	32.62 ± 0.611^{E}	81.75 ± 0.542^{C}	118.75 ± 0.932^{C}	147.62 ± 0.473^{B}
T2	44.62 ± 0.498^{C}	91.62 ± 0.546^{B}	122.75 ± 0.440^{B}	102.75 ± 1.116^{E}
T3	64.75 ± 0.494^{A}	53.62 ± 0.865^{E}	91.62 ± 0.961^{E}	148.125 ± 0.843^{A}
T4	37.87 ± 0.413^{D}	67.37 ± 0.467^{D}	101.75 ± 0.767^{D}	136.75 ± 0.917^{C}
LSD	1.865	6.652	3.214	1.004

The results represented as mean ± SEM.

Different latters denote the presence of significant differences ($P<0.05$) between groups.

C: female rats drenched orally with drinking water (1 ml).

T1: female rats drenched orally with thyroxine (300 mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.

T2: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.

T3: female rats drenched orally with thyroxine and treated with allicin nano particles (50mg/kg of B.W suspended in 1 ml of drinking water) for 30 days.

T4: female rats drenched orally with thyroxine and treated with methimazole (2.5 mg/kg of B.W) and allicin nanoparticles(50 mg /kg of B.W)for 30 days.

Discussion

The results showed a significant decrease in body weight in the T1 group, which received thyroxine alone, compared to the control group. On the other hand, the T2, T3, and T4 groups, which received thyroxine along with Methimazole and Allicin nanoparticles, showed a significant increase in body weight compared to the T1 group. Among these groups, the T2 group had the highest body weight. Improved thyroid function compared to those who received a placebo Additionally, the results demonstrated by (19) demonstrated that rats induced hyperthyroidism by thyroxine treatment lost weight. This substantial decline may be attributable, in part, to the fact that a high thyroxine concentration raises the basal metabolic rate, which in turn raises carbohydrate intake, protein breakdown, and fat consumption. Consequently, the majority of the body's fat deposits go and the weight decreases (20,21) found that the hyperthyroidism group's genetic makeup was significantly less common. The thinking behind this is that when thyroid hormones are released, they speed up important processes, cause heat, and release free radicals. These radicals help denaturate proteins and lipids. The thyroid gland speeds up vital

processes, generates heat, and releases free radicals. Lipids and proteins are broken down by these radicals. The efficacy of catecholamines in fat breakdown is enhanced by thyroid hormone activity, which directly boosts the adenylate cyclase enzyme. According to (22), this process speeds up lipolysis in adipose tissue by promoting adenylate cyclase. The group that took both thyroxine and methimazole, an antithyroid medication, had the most significant weight gain. Weight gain occurred with the combination of thyroxine and allicin nanoparticles, however it was less pronounced than with methimazole alone. More proof that allicin nanoparticles could reduce thyroxine's weight-gaining effects has been found in this study (21). In relationships of body weight, the group that received both allicin nanoparticles and methimazole was significantly heavier than the group that received only allicin nanoparticles. Both treatments have the potential to make patients put on weight, so it's possible that they complement one another (23). Our understanding of the metabolic effects of thyroxine has been further complicated by your study, which has provided light on potential interactions between thyroxine and other medications like methimazole and allicin nanoparticles. Additional research is necessary to identify the underlying mechanisms and comprehend the potential treatment implications of these differences (24).In rats, research has shown that isolated fat cells treated with allicin nanoparticles had a higher responsiveness to lipolysis and better sensitivity. Another agent that breaks down fat is catecholamine's. By (25) Research has shown that elevated serum levels of tumor necrosis alpha correlate strongly with hyperthyroidism, which may explain why the hyperthyroidism group experienced a slower rate of weight gain (26). Loss of body mass is caused by an increase in protein catabolism and other variables; the current analysis found that high energy expenditure is connected with elevated serum tumor necrosis factor levels (27,28).

The levels of various antioxidant parameters, including SOD (Superoxide Dismutase), GSH (Glutathione), CAT (Catalase), and MDA (Malondialdehyde), were measured. The results demonstrated significant differences in the antioxidant status between the experimental groups. Specifically, the MDA level showed a significant difference, indicating variations in oxidative stress. Moreover, the levels of GSH, SOD, and CAT also exhibited significant differences between the experimental groups, suggesting alterations in the antioxidant defense mechanisms. Enzymes including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) play an important role in the body's defence against oxidative stress and free radical degradation. Curiously, when comparing these enzymes to MDA, your results show the inverse patterns. Significant reductions in GSH, SOD, and CAT levels were seen after thyroxine therapy (T1), suggesting a possible reduction in antioxidant defences (29). The results of this study are in agreement with previous research (30,31) showing that antioxidant levels (SOD, GSH, and CAT) were

significantly lower and MDA levels were significantly higher in the group of animals treated with thyroxine that caused hyperthyroidism compared to the control group. The enzyme Na+K+ATPase, which helps with the active transfer of potassium and sodium in opposite directions, uses energy from ATP molecules broken down in cells (32). Other metabolic processes include high rates of fat and carbohydrate metabolism, protein catabolism, and the production of carbonyl protein, which is a protein made when enzymes are damaged due to oxidative stress. Oxygen consumption is a risk factor that leads to increased oxidation within cells and an increase in the amount of free radicals, which in turn leads to the oxidation of many southern molecules (33). By administering methimazole (T2), the thyroxine-induced increase in malondialdehyde (MDA) was reversed, and the antioxidant enzymes that had been depleted were restored to levels that were much greater than those seen in the thyroxine group. This data points to Methimazole's potential anti-oxidant properties in this setting. When compared to the control and thyroxine groups, allicin nanoparticles (T3) showed antioxidant capability as well, with significantly lower MDA and higher GSH, SOD, and CAT levels. These results provide more evidence that the garlic component allicin has antioxidant effects. The body's restricted capacity to detoxify reactive intermediates and an imbalance of ROS are the outcomes of tax stress which is followed by high oxygen consumption, which raises the risk of increased oxidation within cells and a high concentration of free radicals, which oxidizes many essential molecules it also results in DNA damage and lipid peroxidation, which are caused by an imbalance between oxidants and antioxidants, as a result of the high level of oxidants Reduced antioxidant levels and oxidative stress, which causes an imbalance between reactive oxygen species (ROS) and the body's essential capacity to apart from DNA damage and lipid peroxidation brought on by an unbalanced composition of oxidants and antioxidants, the elevated concentration of oxidants also causes it is accompanied by a decline in antioxidants, an oxidative stress condition that creates an imbalance between ROS and the body's critical capacity to detoxify reactive intermediates, and a drop in antioxidant levels the increase in lipid peroxidation led to the excessive generation of active oxygen species and free radicals that inhibit antioxidants and thus their imbalance the end outcome of the lipid peroxidation process is MDA, which is caused by the oxidation of these fatty acids by the action of free radicals (34). The combined effects of methimazole and allicin nanoparticles (T4) were much more pronounced, with the biggest reduction in malondialdehyde and the most notable increases in all antioxidant enzymes among all groups. That the two treatments work together to reduce thyroxine-induced oxidative stress suggests that they have a synergistic antioxidant effect (35). Highlighted the significance of treating hyperplasia patients as a means of preventing ROS toxicity. After that, the thyroiditis may have subsided due to

Graves' disease and multinodular toxic thyroiditis (Methimazole), which are neutralised by free radicals such as hydroxyl, hydrogen peroxide, and superoxide dismutase. The effectiveness of NADPH enzymes, which are proteins that promote oxidation and are directly related to elevated ROS levels, was impacted by the drug's ability to control thyroid hormone levels, according to research by (36). According to (37), the concentration of these enzymes in the liver provided evidence of this. This may clarify why raising serum antioxidant levels while decreasing MDA levels is counterproductive (38). this is because of its regulatory properties, which boost antioxidant systems by increasing the activity of SOD, CAT, and GSH, blocking ROS-forming enzymes, and activating the nuclear factor α, while simultaneously raising and removing MDA levels. According to (39), the antioxidant defence system is not primarily regulated by the erythroid 2 related factor 2 pathway, which is activated. is believed to be the principal regulator of the antioxidant defence system, since it breaks down oxidants and antioxidant-stimulation, which are believed to be induced by fatty acid consumption, and it encourages the creation of proteins with anti-inflammatory features (40). The intricate relationship among thyroxine, oxidative stress, and antioxidant reactions is better understood . It shows that of Methimazole and allicin nanoparticles have the ability to reduce oxidative damage caused by thyroxine; moreover, when these two substances are combined, they have a synergistic impact. We can create antioxidant-based techniques for controlling thyroxine-related disorders if more study investigates the underlying processes and possible long-term ramifications of these results. The lipid profile, consisting of Cholesterol, Triglyceride, HDL (High-Density Lipoprotein), and LDL (Low-Density Lipoprotein), was assessed. The results indicated significant differences in lipid profiles among the experimental groups. Cholesterol and Triglyceride levels exhibited significant variations, suggesting changes in lipid metabolism. Additionally, HDL and LDL levels also showed significant differences, indicating potential alterations in lipid transport. Cholesterol reduction is due to a combination of factors, including the protein that regulates glycerides and a slowdown in the digestion and absorption of cholesterol. According to (41). Due to the fact that the rate of catabolism is higher than the rate of construction, the body's store of most fats decreases, and serum cholesterol levels drop as a result of bile's excretion of cholesterol, this could be harmful to hyperthyroidism caused by thyroxine, which speeds up the pace of food metabolism while also lowering cholesterol (42). Low levels of good cholesterol (HDL), triglycerides (LDL), and antioxidant activity were found in the serum of animals with hyperthyroidism. Oxidative stress, the main culprit, may have led to an upsurge in lipid peroxidation and the generation of free radicals, especially reactive oxygen species (ROS), which could explain the decline in HDL levels and the decline in antioxidant activity (31). The LDL route is the principal regulator

of lipid metabolism, however it is well-known that thyroid dysfunctions are linked to changes in the lipid profile hormone (43). Among all the groups tested, allicin nanoparticles (T3) showed the most lipid-lowering impact, with significantly reduced levels of cholesterol and triglycerides. Allicin, a chemical found in garlic, is known to modulate lipids, and this proves it. The thyroxine treatment (T1) considerably reduced HDL levels compared to the control group, which may raise concerns about atherogenic risk; however, the combined effects of methimazole and allicin nanoparticles (T4) reduced cholesterol and triglycerides even further than T1 and T2, although not to the same extent. This discovery calls for more research into the relationship between LDL and HDL ratios and other variables. T2 methimazole significantly raised HDL levels compared to T1 and reversed the HDL decline generated by thyroxine. Methimazole may help keep HDL levels stable when using thyroxine, according to these results (44). Study (lowering total cholesterol, lowering LDL-cholesterol) According to studies conducted by (45), there was a decrease in both LDL cholesterol and total cholesterol. These benefits were further explained by (46) and further study by (47). For a meta-analysis of randomized clinical trials to determine garlic's antihyperlipidemic effectiveness, further studies need be conducted to investigate HDL cholesterol. According to (48), a little increase in HDL cholesterol in the garlic group was not significantly different from the placebo group. Our search might support the idea that Nano allicin has an effect on elevating HDL cholesterol, and a prescription for garlic pills did lower triglyceride levels (49). Once again, showing the highest levels across all groups, allicin nanoparticles (T3) demonstrated the most substantial favorable influence on HDL. This further supports allicin's promise as an all-natural HDL. T4, which was a combination of methimazole and allicin nanoparticles, reduced HDL levels more than T2 and T3 . Treatment with thyroxine (T1) increased low-density lipoprotein (LDL) levels relative to the control group, which raises additional concerns regarding the possibility of atherogenic risk. People on thyroxine should have their lipid profiles monitored closely because of this discovery. In contrast to T1 and the placebo group, methimazole (T2) effectively decreased LDL levels and reversed the rise in LDL caused by thyroxine. This provides more evidence that Methimazole helps reduce the LDL-raising effects of thyroxine (50). In comparison to T1, T2, and the control group, allicin nanoparticles (T3) had a much less impact on low-density lipoprotein (LDL) and high-density lipoprotein (HDL). This indicates that allicin does not appear to worsen the LDL-raising impact of thyroxine, even if it does not directly reduce LDL. The levels of cholesterol (LDL) were much higher in the T4 group (which included methimazole and allicin nanoparticles) than in T2 while they were marginally lower than in T1. This provides more evidence of the therapies' intricate interaction and calls for additional study into their synergistic impact on

LDL levels. As previously mentioned, it is essential to address restrictions such as the research period, animal model employed, and sample size. Results may be more solid and applicable to a wider population if the trials were longer and included more people (33).

Conclusion:

In conclusion, the use of thyroxine at dose 300mg/kg of B.W 28 days in female rats for hyperthyroidism induction has negative effect and caused imbalance in oxidation – Antioxidants homeostasis. The utilization of Allicin nanoparticles at dose 50 mg/kg of B.W appears to enhance its bioavailability and effectiveness combating oxidative stress associated with hyperthyroidism.

Conflict of Interest: there is no conflict of interest**.**

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